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Original Articles

INDUCTION OF ACUTE GASTRIC LESIONS BY HISTAMINE LIBERATORS IN RATS

EFFECTS THEREON OF PHARMACOLOGIC BLOCKING AGENTS

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HISTAMINE has been given with varying success as a means of evoking gastric ulcer.[1] The most effective mode of administration consists of giving it in a mixture with beeswax and mineral oil[2]; the resultant slow release simulates a state of sustained excessive endogenous histamine liberation. It occurred to us that agents classed as "histamine liberators"[3] might be used advantageously to demonstrate a pathogenic role of endogenous amines in gastric ulcer. Evidence for this hypothesis was found in the lesions described by Smith[4] as occurring in some cats given Compound 48/80 and in the preliminary report of Haverback and Bogdanski[5] on the ulcerogenic properties of reserpine.

During recent experiments on the pharmacology of reserpine and of histamine liberators, we noted a high incidence of gastric ulcers in rats given Compound 48/80 (2.5 to 5 mg. per kilogram) or polymyxin B (2 mg. per kilogram) by intraperitoneal injection. Since our supplies of Compound 48/80 were not sufficient to conduct a series of experiments such as those described below, and because responses to polymyxin B resembled those evoked by Compound 48/80, the experiments reported will be limited to those in which polymyxin B, the more active of the two agents, was used. The aim of these experiments was to demonstrate the nature of the gastric lesions evoked by polymyxin B and to attempt an analysis of their mechanism by administration of drugs which are known to block some of the pharmacologic effects of naturally occurring amines.

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EXPERIMENTAL DATA

1. *Effects of Polymyxin B.*—The first experiments were performed to test the systemic and gastric effects of this agent and the process of healing which takes place after discontinuation of treatment. Sprague-Dawley rats, each weighing 200 to 250 grams, were divided into three groups of twelve animals each. Treatment in each group consisted of a single intraperitoneal injection of polymyxin B in doses of 2, 1, and 0.5 mg. per kilogram of body weight, respectively. The rats had free access to water during the experimental period, thus reducing the immediate mortality from shock.[6] Within minutes after the injection the animals were in shock, prostrate, cyanotic, and dyspneic; the intensity of these symptoms varied in proportion to the dose administered. About half an hour later, while the animals were still semicomatose, they started to drink avidly and some improvement in their condition was noted, particularly in those receiving the smallest dose. Some of the animals showed lenticular opacification. Recovery was slow and was still incomplete seven hours after injection, when six animals from each group were killed.

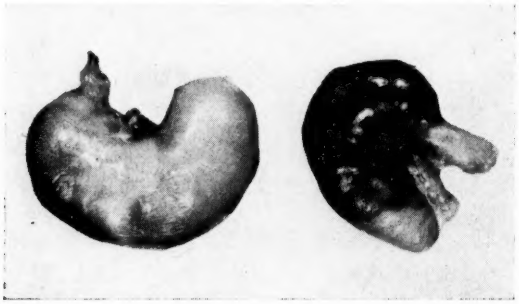

Fig. 1.—Hemorrhagic ulcerations in the great curvature of the stomach and in most of the appendix.

The volume of blood that could be withdrawn from the carotid artery was low, approximating one-half the normal bleeding volume. At autopsy, the stomachs were greatly distended and full of water. It should be noted that the rat's stomach consists of two parts—(1) a glandular area and (2) a proventriculus or rumen which, like the esophagus, has a squamous epithelium. Large, dark, purplish areas, located exclusively in the glandular part of the stomach and sometimes involving the whole area, were visible through the serous surface (Fig. 1). When these were few in number, they were limited to the greater curvature. Besides these lesions, hemorrhagic ulcerations were seen in the part of the cecum which corresponds to the human appendix (Fig. 1), and areas of intense congestion were found in the lungs of some animals. The latter were quite different from those caused by aspiration of blood during sacrifice.

When the stomach was opened, blood was found mixed with gastric content of food and water. The glandular mucosa showed hemorrhagic erosions.

In these areas the superficial epithelium was loosely adherent to the subjacent structure from which it was detached when the stomach was washed. The area of these lesions relative to the total glandular area, which can be considered an index of their severity, and their incidence are shown in Table I. Doses of 2 and 1 mg. per kilogram of body weight caused lesions in all animals. The extent of the lesions was less in those given the smaller dose, however, while both severity and incidence decreased sharply in those given only 0.5 mg. per kilogram.

TABLE I. EFFECTS OF POLYMYXIN B ON INCIDENCE AND SEVERITY OF GASTRIC LESIONS

	POLYMYXIN B (MG. PER KG.)		
	2	1	0.5
Per cent incidence	100	100	16
Per cent severity*	43	17	1

*Involved area expressed as a per cent of the total glandular area.

Microscopic examination showed extensive or well-circumscribed areas of hemorrhagic necrosis. Those lesions which were isolated and small occurred between the rugae of the greater curvature. In some cases they were superficial, representing mere erosions (Fig. 2); in others they extended through to the muscularis mucosa, so that only a few epithelial cells remained. These areas showed no signs of inflammation or deposition of fibrin. Edema, if present, was slight and occurred only with the deeper lesions. The blood vessels were dilated but morphologically intact. Hemorrhages were confined to the mucosa, being limited by the muscularis mucosae. Thus, these fulminant lesions seem to result from an altered circulation and superficial hemorrhages, the extent of the necrosis varying with the depth of the hemorrhagic zone.

The remaining six animals of each group were killed from one to four days after the single injection of polymyxin B. At this time they had completely recovered from shock, the cataracts had cleared, and they showed no gross ill effects of treatment. At autopsy, the stomach walls were markedly edematous. From microscopic examination, the evolution of the lesions was visualized to proceed as follows: First, the necrotic epithelium had sloughed off, leaving in the most severe cases a bare layer of muscularis mucosae covered by fibrin; prompt regeneration of epithelium was evident in those lesions which showed little or no inflammatory reaction, so that by the fourth day areas of erosion had developed a complete epithelial surface with regenerated but, as indicated by PAS stain, not secreting glands. In the more severe cases, the base of the ulcers showed an inflammatory reaction with polymorphonuclear infiltration and edema extending through to the muscularis (Fig. 3). The blood vessels were still dilated but otherwise normal.

In an attempt to evoke more severe lesions which might go on to perforation, a group of animals were given three successive increasing doses of from 2.5 up to 7.5 mg. of polymyxin B during a period of three days. Tolerance was demonstrated by the fact that the symptoms caused by the second and third doses were not as severe as those resulting from the first. Animals were

killed on the fourth day. Two types of lesions were seen, namely, old lesions caused by the first injection and already in process of repair and fresh lesions resulting from the additive effects of the three injections. These lesions correspond to those described above (epithelial hemorrhagic necrosis, fibrin deposition, inflammation, and edema), except that vascular damage was more severe. Hemorrhages extended through the muscularis mucosae to the markedly edematous submucosa (Fig. 4). Vascular lesions in the arterioles and arteries were common. Eosinophilic material was deposited in and around the blood vessel walls, spreading into the surrounding connective tissue; this material stained red with periodic acid-fuchsin stain (PAS) and dark blue with phosphotungstic acid-hematoxylin stain (PTAH), suggesting that it is fibrin (Fig. 5). The endothelium of these vessels was edematous, protruding into the lumen. The muscularis showed hydropic degeneration and focal necrosis in the fibers near the damaged submucosa. No instances of perforation were found. In neither these nor the previous experiments were ulcerations seen in the duodenum, although they were common in the cecum.

2. *Effects of Pharmacologic "Blocking" Agents on the Production of Gastric Lesions.*—The major compounds released by amine liberators are histamine, serotonin, and noradrenaline. Hence, we studied the effects of antihistaminic and serotonin-blocking agents and of an adrenolytic drug in polymyxin-treated rats.

Lysergic acid diethylamide (LSD), a potent antiserotonin agent[7] was injected intravenously in a dose of 100 mcg. per kilogram. Chlorpromazine which has weak adrenolytic, antiserotonin, and antihistaminic properties,[8] was used since it inhibits the dextran reaction[9] and the pressor effect of reserpine[10] in the rat; it was given in subcutaneous doses ranging to 10 mg. per kilogram. The antihistaminics tested were Phenergan (promethazine), Thephorin (phenindamine), Chlor-Trimeton (chlorprophenpyridamine), and Dimetane (parabromdylamine); these were given in subcutaneous doses of 20 mg. per kilogram. Dibenzyline (phenoxybenzamine), which is mainly an adrenolytic drugs, was used because it had been found to inhibit responses to reserpine in iproniazid-treated rats;[10] it was administered intravenously in a dose of 1 mg. per kilogram. Each drug was given one-half hour before the intraperitoneal administration of a standard dose of 2 mg. of polymyxin B per kilogram. The effects in these animals were compared with those of a control group given polymyxin B alone.

Shock was prevented by the antihistaminics (with the exception of Dimetane), chlorpromazine, and Dibenzyline. Shock occurred in the Dimetane-treated group, but it was transient, passing off within two hours. The only gross disturbance in those animals protected from shock was hyperemia of the extremities, which compared with the cyanosis present in the controls and in unprotected test animals. Chlorpromazine also prevented shock, but the protection was less evident than in other groups, largely because of the sedation induced by this agent.

Results in terms of gastric ulceration and details of the procedures are summarized in Table II. These show that only the antihistaminics and Dibenzyline afforded protection from gastric erosions. LSD and chlorpromazine

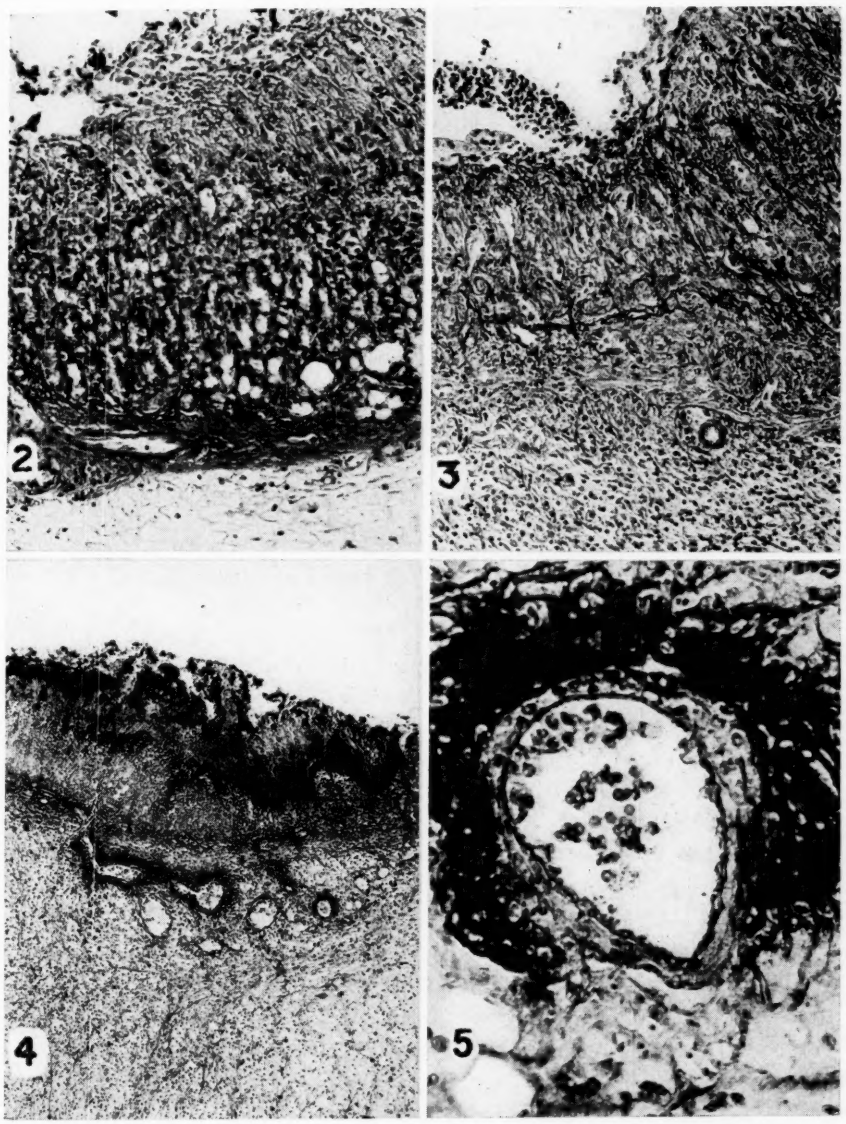

Fig. 2.—Section of the stomach showing a superficial ulcer. Half of the mucosa is necrotic. Glands and blood vessels are dilated. There is no inflammatory reaction, but slight edema is evident. (Periodic acid-fuchsin stain. Magnification, ×100.)

Fig. 3.—Ulcer in the process of healing after sloughing of the necrotic part; inflammation in the submucosa. (Periodic acid-fuchsin stain. Magnification, ×125.)

Fig. 4.—Acute necrosis and inflammation of the mucosa down to the muscularis mucosa. The submucosal connective tissue under the necrotic area shows edema, red blood corpuscles, and inflammatory cells; blood vessels are dilated and surrounded by material which takes up the periodic acid-fuchsin stain. (Periodic acid-fuchsin stain. Magnification, ×45.)

Fig. 5.—The same specimen as shown in Fig. 4, showing fibrinous exudate and edema around a small artery. (Phosphotungstic acid-hematoxylin stain. Magnification ×370.)

had no significant effect on the severity and incidence of gastric ulcerations. In order of decreasing activities, the antihistaminics were Phenergan, Chlor-Trimeton, Thephorin, and Dimetane. Dibenzyline was as active as Phenergan in protecting against erosions.

TABLE II. EFFECTS OF VARIOUS BLOCKING AGENTS ON ULCER-INDUCING PROPERTIES OF POLYMYXIN B

TREATMENT*	NO. OF RATS	PER CENT MORTALITY	ULCERS (PER CENT INCIDENCE)	PER CENT SEVERITY
Control	22	40	100	52
Phenergan 20 mg.	22	0	0	0
Chlor-Trimeton 20 mg.	6	0	17	1
Thephorin 20 mg.	6	0	83	7
Dimetane 20 mg.	6	0	83	17
LSD 100 mcg.	6	83	100	66
Chlorpromazine 10 mg.	6	17	100	46
Dibenzyline 1 mg.	6	0	0	0

*All the rats received one intraperitoneal dose of 2 mg. per kilogram of polymyxin B. All doses given are expressed per kilogram of body weight.

3. *Effects of Other Amine Liberators on the Ulcerogenic Action of Polymyxin B.*—Reserpine is known to evoke gastric ulcerations in rats[5]; these have been attributed to release of serotonin. While LSD was inactive in the experiments described here the possible participation of serotonin in the reaction to polymyxin was further explored. Reserpine is known to cause a prolonged depletion of most of the body stores of serotonin[5, 11] and to inhibit the egg-white reaction[12]; hence, ulceration consequent on administration of polymyxin B to animals pretreated with reserpine should not be attributable to release of serotonin. A second agent, dextran, was also studied since it is not itself ulcerogenic but is known to release both serotonin and histamine from body stores.[14]

The study is summarized in Table III. In brief, forty-eight rats were divided into three groups: Group I served as control; reserpine pretreatment (3 mg. per kilogram, forty-eight hours before injection of polymyxin B) was given in Group II; and dextran pretreatment (300 mg. per kilogram twenty-four hours before polymyxin B) was given in Group III. The animals that received polymyxin B (2.5 mg. per kilogram) were sacrificed seven hours after injection, while those given reserpine and dextran alone were sacrificed after twenty-four hours. Reserpine provoked drowsiness, piloerection, salivary hypersecretion, and defecation; dextran caused only hyperemia and edema of the nose and extremities; polymyxin B elicited shock and death in one-half of the group. When polymyxin B was given to rats pretreated with reserpine, shock did not occur and the extremities were hyperemic rather than cyanotic. When polymyxin B was given to rats penetrated with dextran, the symptoms usually caused by polymyxin B were alleviated; shock did not occur, and

there were only moderate dyspnea and cyanosis in some of the animals. Examination of the stomach showed that dextran alone caused no lesions and that reserpine caused punctiform erosions which were insignificant as compared with those evoked by polymyxin B. Reserpine did not affect the post-polymyxin erosions, whereas dextran reduced their severity but not their incidence.

TABLE III. MODIFICATION OF TOXIC EFFECTS OF POLYMYXIN B BY RESERPINE AND DEXTRAN*

TREATMENT	POLYMYXIN B 2.5 MG.	RESERPINE 3 MG. + POLYMYXIN B 2.5 MG.	DEXTRAN 300 MG. + POLYMYXIN B 2.5 MG.	RESERPINE 3 MG.	DEXTRAN 300 MG.
No. of rats	12	12	6	12	6
Per cent mortality	50	0	0	0	0
Ulcers					
Per cent incidence	100	100	85	25	0
Per cent severity	77	64	13	1	0

*All doses are expressed in milligram per kilogram of body weight.

In experiments described elsewhere,[10] we noted that in the rat pretreatment with iproniazid potentiates the pressor effects and alters the symptomatic effects of reserpine. Therefore, we could presume that by inhibiting the rate of destruction of amines liberated by reserpine, iproniazid should increase the incidence and severity of ulcers caused by reserpine and attributable to release of serotonin. With this in mind, tests were done on the effects of reserpine (3 mg. per kilogram) in rats pretreated with iproniazid (50 mg. kilogram), some of which were also given Dibenzyline as described above. In brief, iproniazid pretreatment did not alter the degree of ulceration significantly but did evoke pulmonary edema after reserpine injection. This pulmonary edema was presumably due to potentiation of the action of adrenergic amines, since it was prevented by Dibenzyline. This experiment would suggest that iproniazid inhibits destruction of adrenergic amines to a greater extent than it does that of serotonin.

DISCUSSION

Apart from the light they may shed on the possible pathogenic role of endogenous amines in the genesis of gastric erosions and appendiceal lesions, these experiments also clarify the nature of some of the so-called "toxic" effects of polymyxin B and demonstrate that these may be suppressed by adequate dosages of antihistaminics and adrenolytic agents.

The method described also provides a new technique for the production and study of healing of gastric ulcers. It is, as compared with the histamine-beeswax procedure,[2, 15] rapid and predictable. As compared with the Shay procedure of pyloric ligation,[16] it corresponds more closely to physiologic conditions, and, since the ulcers provoked by polymyxin B are in the glandular part of the stomach and not in the squamous proventriculus, they are comparable to those that occur clinically. This new procedure complements the Shay technique, since the two types of ulcers seem to depend on different basic mechanisms.

The experiments indicate the nature of the ulcerogenic agents released during the action of polymyxin B. Of these, the most important is evidently histamine, as evidenced by the protective action of antihistaminics. The nature of the lesions suggests that histamine acts primarily by disturbing the circulation of the gastric submucosa rather than by inducing acid secretion. Although antihistaminics can inhibit the gastric acid and pepsin hypersecretion caused by histamine,[15, 17] it is doubtful that this explains their antiulcerogenic properties.[15] It is interesting that from studies on acid secretion caused by histamine as well as the present experiments, Phenergan was found to be the most potent antihistaminic.[17] The experiments therefore point to a possible new mode of assay of the relative potency of antihistaminic drugs, based on their protective effect against polymyxin B ulceration, which would complement those in present use and would have more direct therapeutic applications.

The possibility that serotonin participates in this syndrome seems remote. This statement is based on the following considerations: (1) polymyxin B is known to release substantial quantities of histamine and little serotonin from body stores, while Compound 48/80, which is a less potent ulcerogenic agent than polymyxin B, causes release of both histamine and serotonin in roughly proportionate amounts[6]; (2) reserpine, which depletes body stores of serotonin, does not affect the incidence or severity of ulceration consequent on polymyxin B, while dextran, which causes release of both histamine and serotonin, does offer some protection, presumably because of histamine depletion[14]; (3) in this regard, LSD and chlorpromazine, both of which act as serotonin antagonists, do not affect the lesions evoked by injection of polymyxin B.

The probability that adrenergic amines may participate in the ulcerogenic effects of histamine liberators is suggested by the protective effect of Dibenzyline; it prevents ulcer formation due to polymyxin B, inhibits edema caused by dextran,[13] and prevents the fall in blood pressure which occurs after injection of various histamine liberators.[24] Shock, against which Dibenzyline also protects, is evidently not a factor in ulcer formation, since both reserpine and chlorpromazine—drugs with other similar properties—protected against shock but not against ulceration. It is more likely that the inhibitory effects of Dibenzyline are due to its relatively strong antihistaminic properties.[25]

Since mast cells occur in large numbers in the stomach wall of the rat,[19] it might be postulated that the amines which cause ulceration are locally liberated. Against this is the fact that gastric gavage with either polymyxin B or Compound 48/80, as well as local injection of these substances (1 mg. per kilogram) into the gastric wall, did not provoke ulceration. Presumably, the drugs given by gavage either were not absorbed or were destroyed; those locally injected were devoid of activity in spite of the relatively high concentration of the drug in contact with histamine depots. Hence, it followed that histamine is released from extragastric sources. These observations correspond with those of Smith,[4, 20] which indicate that Compound 48/80 is much less effective in causing histamine release and gastric secretions when injected

into the celiac artery than when given intravenously. Furthermore, the experiments indicate that, to induce a sufficient release of amines from peripheral cells, the amine liberator used must be a very potent one, such as polymyxin B or Compound 48/80, since such agents as ovomucoid and dextran are not ulcerogenic.

The mechanism of ulceration is presumably one of submucosal hyperemia and stasis followed by hemorrhage. That gastric acid secretion is not a factor was indicated by a small group of experiments in which intragastric administration of aluminum hydroxide was found not to protect against polymyxin B ulceration. It has also been postulated by others that the mechanism of ulceration in guinea pigs given histamine is primarily circulatory and due to the effect of histamine on the small vessels of the stomach wall.[21] A circulatory disturbance is generally conceded to be the basis of the Curling ulcers evoked by shock or severe burns,[22] and high histamine blood levels have been observed in this condition.[23] These ulcers also compare with those developing in the first phase of alarm reaction[22] in which there are also prostration and circulatory collapse. In this condition, however, the systemic circulatory defect may be primary since the ulcers, although they occur in the glandular part of the stomach, are punctiform, small, and less diffuse than those resulting from the injection of polymyxin B. Indeed, these ulcers of the alarm reaction correspond with those which have been described as resulting from injection of 5-hydroxytryptophan or reserpine.[5]

SUMMARY AND CONCLUSIONS

1. Injection of a potent histamine liberator, polymyxin B, into rats consistently results in mucosal hemorrhages and erosions or ulcerations of the glandular part of the stomach. Healing begins immediately, and epithelization may be complete in four days. Ulcerations are prevented by potent antihistaminic drugs and by phenoxybenzamine, an adrenolytic compound which also possesses antihistaminic properties.

2. It seems that release of histamine from peripheral body stores is a primary cause of the vascular disturbances which result in gastric lesions. The experiments indicate that serotonin plays no major part in causing this form of ulceration, whereas it may participate in the ulcers provoked by reserpine.

3. This new method of eliciting gastric ulceration provides a technique which is convenient and which complements the Shay procedure. It may serve as a useful basis for assay of relative potencies of antihistaminic drugs.

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ALLERGY IN FUR WORKERS WITH SPECIAL REFERENCE TO PARAPHENYLENEDIAMINE

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IN 1898, Brocq[3] described a patient with eczema caused by hair dye that contained paraphenylenediamine.* In the ensuing fifty years numerous investigators have studied the chemistry,[9, 11, 12, 13, 15, 20, 23] pharmacology,[6] and pathogenic properties[2] of this drug and its oxidation products. It was originally held that the symptoms which resulted from exposure to the drug and its oxidation products were toxic in character.[1, 2, 4, 6, 9, 19] In 1920, Gerdon,[10] on the basis of experiments with guinea pigs, concluded that the symptoms produced were of an anaphylactic nature. This was confirmed by Curschmann[5] in a study of seven patients with asthma and was subsequently supported by others.[8, 11, 18, 23] In 1931, Müller[20] sensitized thirteen nonallergic patients by a daily application of 2 per cent "P.D." in petrolatum. After a period of from two to nineteen days, he was able to obtain positive patch tests not only at the original site but on other parts of the body as well.

MATERIAL AND METHODS

From 1941 to 1955, one of us (D. E. S.) studied eighty fur workers who had been employed in the fur industry and who had developed symptoms referable to the skin, the respiratory tract, or both. There were seventy-four men and six women in the series, and their ages ranged from 18 to 67 years. Most of the patients were equally distributed through the third, fourth, and fifth decades of life when first seen. With few exceptions, the study included a history, physical examination, complete hemogram, urinalysis, chest x-ray, electrocardiogram, and complete intradermal and scratch tests to foods, inhalants, pollens, molds, fur danders, and, when available and necessary, fur-dye dust from the shop in which the patient worked. Patch tests and inhalation tests were done where indicated.

CLINICAL SYNDROMES ENCOUNTERED

Among the eighty patients studied, the largest single diagnostic category was bronchial asthma (Table I). Asthma occurred as the sole symptom in thirty patients (37 per cent, and appeared alone or in combination with other allergic symptoms in fifty-nine patients (74 per cent). Vasomotor rhinitis was the second most frequent clinical entity, and it appeared alone or associated with other allergic symptoms in thirty-eight patients (47.5 per cent). In only five patients (6 per cent) did all of the major symptoms—bronchial asthma, vasomotor rhinitis, and contact dermatitis—appear.

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*Paraphenylenediamine will henceforth be indicated as "P.D."

TABLE I. DISTRIBUTION OF ALLERGIC SYNDROMES IN OUR SERIES

ALLERGIC SYNDROME	NO. OF PATIENTS	PER CENT
Bronchial asthma	30	37
Bronchial asthma with other allergies	29	36
Vasomotor rhinitis	6	7.5
Vasomotor rhinitis with other allergies	32	40
Contact dermatitis	10	12.5
Contact dermatitis with other allergies	8	10
Cough	2	2.5
Cough with other allergies	2	2.5
Bronchial asthma, vasomotor rhinitis, and contact dermatitis in same patient	5	6.2

TIME OF EXPOSURE IN THE FUR INDUSTRY

With the exception of Reichel's[22] single patient who developed symptoms after only two days of exposure in the industry and Straube's[23] patient with twenty days of exposure, most investigators[4, 8] observed that the exposure time varied from three months to ten years. Mayer[14] found that almost 25 per cent of his patients with asthma had been exposed for less than six months.

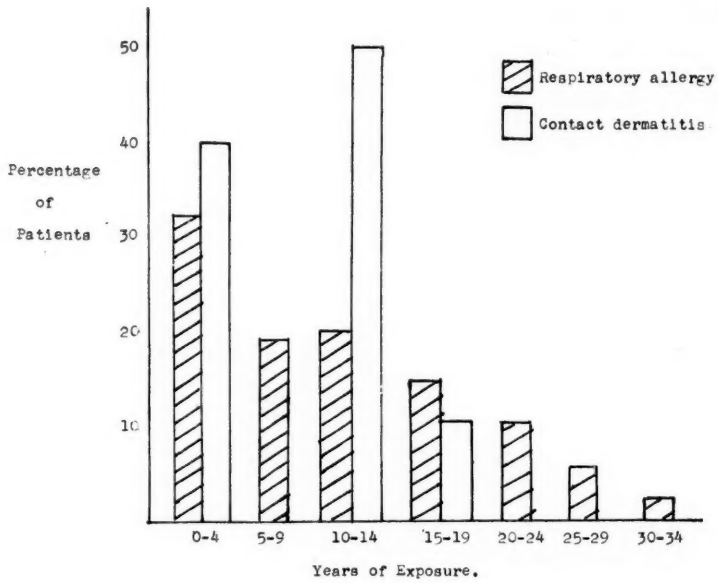

Fig. 1.—Years of exposure in the industry before the onset of allergic symptoms in seventy-seven fur workers.

An analysis of our data confirms the findings that the time of exposure before the onset of symptoms is usually a matter of years. It may be seen in Fig. 1 that, of the sixty-seven patients in our series with respiratory symptoms, only twenty-one (31 per cent) had their onset with less than five years of exposure, and the remainder, or more than two-thirds of the group, required from five to thirty-one years of contact with the offending agent before the symptoms began to appear.

Of ten patients in whom the sole symptom was contact dermatitis, all had been exposed for more than one year and the majority had required contact for from ten to fifteen years (Fig. 1).

PATCH TESTS

Since "P.D." and closely related chemical substances are almost universally employed in the dyeing of furs, patch tests were performed with 2 per cent "P.D." in petrolatum, with 3 per cent "P.D." in aqueous solution, and also with the latter solution diluted with an equal volume of 3 per cent hydrogen peroxide. In certain patients, an extract of dye or dust obtained from their respective places of work was used. A test was not considered to be positive unless either erythema, vesiculation, or both were present, with or without local pruritus. Usually the patch was removed after forty-eight hours, and the reaction was noted. The time required for the reaction to appear varied from several hours to four days.

Among fifty-eight patients with bronchial asthma, patch tests were done in forty-seven, and positive reactions to one or more of the four tests was elicited in thirty-one (65 per cent). The incidence of positive reactions varied with the preparation applied. Materials obtained from the place of employment gave the highest percentage of positive reactions (64 per cent), to the aqueous, peroxide, and petrolatum tests in decreasing order of frequency (Table II).

TABLE II. RESULTS OF PATCH TESTS WITH VARIOUS PREPARATIONS OF PARAPHENYLENEDIAMINE IN FUR WORKERS WITH BRONCHIAL ASTHMA AND CONTACT DERMATITIS

	BRONCHIAL ASTHMA (47 PATIENTS)			CONTACT DERMATITIS (10 PATIENTS)		
TEST SOLUTION	TOTAL NO. OF TESTS	NUMBER POSITIVE	PERCENTAGE POSITIVE	TOTAL NO. OF TESTS	NUMBER POSITIVE	PERCENTAGE POSITIVE
Paraphenylenediamine, 3 per cent aqueous solution	36	19	53	6	6	100
Paraphenylenediamine, 2 per cent in petrolatum	33	12	36	6	5	83
3 per cent "P.D." in aqueous solution in equal volume H_2O_2	21	14	44	6	3	50
Shop dye	11	7	64	4	3	75
Combined total of patients	101	31	65*	22	10	100*

*Percentage of patients who gave positive reactions with one or more of the test materials.

Mayer and Forster,[17] in their study of forty-three patients with asthma due to "P.D.," obtained 30.1 per cent positive reactions using 2 per cent "P.D." in petrolatum, and Silverberg and Heimann[24] found the same incidence in ten patients. Our figure of 36 per cent (Table II) confirms these data. From our study, however, it is not possible to predict which of the three

tests we employed will elicit a positive reaction in any given subject. In our opinion, all three tests and a patch test with shop material should be done in order to obtain the greatest number of positive reactors.

Of the ten patients with contact dermatitis, all reacted positively to at least one of the four tests performed, and all of the six patients tested with 3 per cent "P.D." in aqueous solution gave positive reactions. The high incidence of positive patch tests in patients with contact dermatitis is in accord with the findings of Silverberg and Heimann,[24] who showed that 80 per cent of their patients with dermatitis gave positive patch tests with 2 per cent "P.D." in petrolatum. Mayer and Forster,[17] in their study of fifty patients, found 46 per cent positive reactors. Only twenty-two patients in the latter series, however, had dermatitis at the time of the survey. The incidence of positive patch tests in nonallergic persons and in allergic persons not connected with the fur industry has been amply studied by other investigators using the same techniques that we have employed, and these persons will serve as controls.

In contrast to these observations in fur workers who showed symptoms, Percival[21] found no positive reactors to 2 per cent "P.D." in petrolatum in a study of fifty nonallergic patients. Similar patch tests done by Mayer[16] on a nonsymptomatic group of fur workers who had varying types of contact with the dyeing process showed that the tests were positive in 26.2 per cent of the group directly involved in the dyeing process and in only 11.4 per cent of those who handled the dried dyed fur. There appears to be a correlation between the occurrence of a positive patch test, on the one hand, and both the presence of symptoms and the degree of exposure to the dye, on the other

INTRADERMAL AND SCRATCH TESTS

The substances used in routine testing were the pollens generally found in the New York area, the common inhalants, molds, foods, and fur danders. The results of these tests are shown in Table III. The greatest number of positive reactions was obtained with the inhalants (67 per cent) and with the autogenous dye dust (66 per cent) followed, in order of decreasing frequency, by foods (55 per cent), pollens (41 per cent) and fur danders (25 per cent).

TABLE III. RESULTS OF INTRADERMAL AND SCRATCH TESTS IN OUR SERIES

	POLLENS	FOODS	INHALANTS	FURS	SHOP DUST
Number of patients	70	65	64	63	39
Number of patients positive*	29	36	43	16	26
Percentage positive	41	55	67	25	66

*In each instance the patient gave a positive skin test to at least one allergen in the category.

Earlier, however, Shilkret and Swartz[25] showed that positive intradermal tests with an extract of fur-dye dust occurred with the same frequency in allergic patients within the fur industry as in a control series of allergic patients outside the fur industry.

INHALATION TESTS

In 1929, Genkin and Owtschinski[9] had noted that some of their patients with asthma due to "P.D." reacted with sneezing when exposed to "P.D." powder or fumes. In 1931, Reichel[22] obtained positive inhalation tests on both of his "P.D." asthma patients. Straube[23] produced an asthmatic attack in his patient with "P.D." asthma four hours after spraying "P.D." powder and hydrogen peroxide in an "asthma chamber."

Whenever it was feasible, inhalation tests were done on patients who had symptoms referable to the respiratory passages. Either 3 per cent "P.D." in aqueous solution, the former mixed with an equal volume of 3 per cent hydrogen peroxide, or extracts prepared from shop dusts and furs were used. One or more of these tests might have been performed on the same patient at different times. The solutions were placed in a DeVilbiss No. 40 nebulizer, and the patient was asked to inhale the aerosol produced. The inhalation was by the oral route, unless it was necessary to test the nasal mucosa. In the latter instance, the nose was the route of inhalation. The tests were done when the patient was asymptomatic. Positive reactions were elicited in about one minute; at times the latent period extended to several hours. A reaction was deemed to be positive when one or more of the symptoms of sneezing, rhinorrhea, tightness in the chest, persistent cough, wheezing, or dyspnea appeared. That the response is not due to the action of the test material as a primary irritant is demonstrated by the absence of reactions in numerous other patients.

TABLE IV. RESULTS OF INHALATION TESTS IN FUR WORKERS WITH RESPIRATORY SYMPTOMS

SUBSTANCE INHALED	NUMBER OF PATIENTS	NO. OF TESTS POSITIVE	PER CENT POSITIVE
Paraphenylenediamine, 3 per cent aqueous solution	20	11	55
3 per cent "P.D." aqueous solution in equal volume 3 per cent H_2O_2	24	12	50
Shop dye	34	28	82
One or more of above tests done	50	—	74*

*Percentage of patients who gave positive reactions with one or more of the test materials.

Thirteen (26 per cent) of the patients with an indisputable diagnosis of allergic respiratory disease related to "P.D." exposure established by other compelling clinical criteria failed to give a positive reaction to the inhalation test. As shown in Table IV, the highest percentage of positives (82 per cent) was obtained when shop dye material was inhaled. Dye preparations gave positive reactions in at least 50 per cent of the patients tested. Thirty-seven (74 per cent) of the patients tested by inhalation were positive to one or more of the dye substances employed.

POLLINOSIS

In this series of eighty patients, there were nine (11 per cent) with clinical hay fever (one of whom was negative to the usual skin tests). Of seventy

patients who were tested with pollens, twenty-nine (41 per cent) gave positive reactions. The unusually high incidence of hay fever and of positive skin reactions to pollen extracts raises the question as to whether or not this points to an inheritance factor for allergy in this group. Unfortunately, we do not have the data to determine the age at onset of the pollinosis in relation to the employment; nor did we plan a control group of "P.D"-nonsensitive fur workers. We believe, however, that the conditions are related in that, regardless of which appeared first, it set up local conditions in the respiratory mucosa in the individual worker which made it easier for the second illness to develop.

EOSINOPHIL STUDIES

Eosinophil counts were done on forty-nine patients in this series, and in thirty-three of them the range was from 0 to 4 per cent (normal). In the remaining sixteen, the eosinophil counts ranged from 6 per cent to 32 per cent. One patient had vasomotor rhinitis (8 per cent eosinophils), whereas the remaining fifteen had asthma alone or in combination with vasomotor rhinitis or contact dermatitis. The absence of eosinophilia in our patients who had only dermatitis is probably due to the relatively brief duration of the illness in most instances.

DIAGNOSTIC CRITERIA

Forker,[8] on the basis of clinical studies, stated that a positive clinical history with a positive patch test to "P.D." was evidence that the illness was due to "P.D." sensitivity. We feel that the most conclusive evidence for the existence of respiratory allergy due to "P.D." or related substances is the demonstration that there are a positive clinical history, a positive inhalation test, and a cure by removal from environment. On the other hand, failure to elicit a positive inhalation test does not warrant the conclusion that "P.D." is not the responsible allergen. It does demonstrate, however, that the inhaled test material is not a primary irritant. In the latter circumstance, an especially careful scrutiny of other possible offending agents at the place of work, particularly chemicals and other nonoccupational inhalants, is indicated. There remains a group, however, which, on the basis of careful history and exclusion tests, is clinically sensitive to "P.D." Possible reasons for the negative inhalation test are inability to duplicate the occupational environment (especially with regard to duration of exposure,) and nonspecific irritants, such as dusts and chemicals.

TREATMENT AND PROGNOSIS

Treatment, especially in those patients who had respiratory symptoms, was directed not only toward hyposensitization by injection with an extract of the shop fur-dye dust but toward elimination of offending foods, molds, and inhalant substances and administration of the standard therapy for pollen and house-dust sensitivity when this was present. In addition, many patients received nonspecific treatment with vaccine. A number of these patients were persuaded to wear face masks at work. The latter device enabled a few to

remain on the job. There was no evidence that parenteral therapy, such as injections with fur-dye dust, house dust, vaccines, or pollen in those with hay fever, or the elimination of offending foods (by skin test) influenced the symptoms that were caused by sensitization to "P.D." Only eleven patients were not under observation and came in merely for diagnostic evaluation. These patients all had contact dermatitis and all of them were cured on removal from the work environment. The remainder, or sixty-nine patients, were under close scrutiny and treatment for a period of from one month to six years, with an average period of observation of twenty months. Over 75 per cent of the patients were observed for a period of six months or more.

The only effective approach was complete avoidance, such as may be achieved by a change of occupation. The overwhelming majority of patients who showed respiratory symptoms due to "P.D." were completely "cured" by ceasing their work in the fur industry. Most of the patients were observed over periods of time varying from months to years. Each time they returned to work with furs, the symptoms reappeared. The clinical criteria for the etiological relationship between symptoms and exposure to "P.D." are as compelling as those employed in the diagnosis of ordinary seasonal pollinosis. The prognosis for patients with respiratory sensitization is excellent if they remain away from the fur industry or other industrial exposure to the dye. In only four instances was complete avoidance not followed by abatement of symptoms. With the foregoing exceptions, none of our patients experienced a return of symptoms. This seemed to occur in those patients with a longer duration of illness. In a few patients with dermatitis, the lesion could be controlled by the use of protective skin creams.

In accordance with our experience, von Criegern,[4] in 1902, and Curschmann,[5] in 1921, noted that the most effective therapy was that the patient quit the industry. Several countries, among them England, France, and Germany, have attempted to introduce legal codes of a prophylactic nature that have as their basis the minimizing of contact with the dye, thus re-emphasizing the great importance of the quantitative factor in the etiology of the acquisition of sensitization and in its perpetuation. Feil,[7] in 1940, indicated the need (1) to use another type of fur dye, (2) if this is not possible, to use the most dilute solution that is effective, (3) to wear protective garments (gloves, etc.), (4) to rinse hands as frequently as possible, (5) to urge sensitized workers to change occupation, and (6) to test new workers in the industry with "P.D."

CONCLUSIONS

1. About two-thirds of the patients studied required five or more years of exposure to the dye before symptoms began to appear.

2. There is a high degree of correlation between a positive clinical history of paraphenylenediamine sensitization and the occurrence of a positive patch test to that substance.

3. In this series, all patients with contact dermatitis as the sole symptom gave a positive patch test.

4. The incidence of positive patch tests to paraphenylenediamine rises with an increase in the variety of preparations of that substance used in testing.

5. The most reliable evidence of respiratory sensitivity to paraphenylenediamine is a positive inhalation test to the substance.

6. In this series of patients with allergy to paraphenylenediamine, 11 per cent had clinical hay fever.

7. No therapeutic approach other than change of occupation appears to be effective.

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THE RELATIONSHIP BETWEEN BRONCHIOLITIS AND CHILDHOOD ASTHMA

A Follow-up Study of 100 Cases of Bronchiolitis in Infancy

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"IF YOU wish to converse with me, define your terms." This Voltarian requirement for an intelligent discussion has caused various authors a fair amount of difficulty in the case of the disease entity in question. The diagnosis of acute bronchiolitis in the living child is often enough fraught with certain vaguenesses, and its definition is not at all identical in the texts consulted.[6-8, 10] An indication of the divergence of opinion may be found in the nature of synonyms assigned to this condition—acute capillary bronchitis, acute asthmatic bronchitis, and acute interstitial pneumonia.

Whenever the microscopist demonstrates thickened and infiltrated bronchiolar walls in a small infant who died with symptoms of acute obstructive emphysema, very few will question the diagnosis of acute bronchiolitis. There also seems to be a fair amount of agreement concerning the statement that bronchiolitis is an acute infectious disease. Less agreement exists with regard to the causative agent. Viral, specific, or nonspecific bacterial infections, or combinations of these, have been considered "the" cause. It is somewhat difficult to understand how the clinical symptoms of such a definite pathologic and clinical entity can be produced equally well by a variety of other etiological factors.[1-6, 7, 9, 12]

Aside from the pathologic description, a number of clinical signs seem to be rather unanimously accepted as criteria for the diagnosis of bronchiolitis in infancy.[7, 12] These include low-grade fever, sibilant musical râles with or without associated fine moist râles, a hyperresonant chest, inconclusive hematographic findings, and roentgenographic signs of obstructive emphysema with or without scattered parenchymal infiltrations.

We have been guided by this group of criteria in selecting the cases for the study presented here. Unfortunately, it was necessary in most instances to depend upon the accuracy of hospital records.

MATERIALS AND METHODS

Between 1950 and 1957, a total of 12,600 children were admitted to the pediatric services of three major hospitals of Rochester, New York—the Genesee Hospital and the Strong Memorial-Rochester Municipal Hospitals. Of

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these 12,600 children, 182 (1.4 per cent) met the usually accepted criteria for the diagnosis of acute bronchiolitis.

In January, 1958, we proceeded to attempt follow-up studies on these 181 cases by either contacting their private physicians or consulting the records at the various hospitals in the case of service patients. One hundred of the 181 children could be traced.

The study attempted to ascertain two possibilities:

1. Whether or not the patients in question had developed a major respiratory allergy since discharge from the hospital. The diagnoses sought were asthma, perennial allergic rhinitis, recurrent upper respiratory disorder, and pollinosis (hay fever).
2. Whether or not the family history showed any incidence of either asthma, hay fever, or eczema (atopic dermatitis) in the immediate family. (These were considered to be commonly recognized diseases which could be ruled in or out by interview.) The family history was obtained by making telephone contact with or by writing to the patient's family.

RESULTS

Of 100 patients successfully traced, forty-three were clinic patients, while fifty-seven were followed by their private physicians, usually a pediatrician or general practitioner in the Rochester area. The youngest patient with bronchiolitis was 4 weeks old on admission, and the oldest was 4 years old; 76 per cent were under 1 year of age. The interval between the admission and the time of this study was between twelve months and seven years in all but three cases. These latter three patients were admitted in early 1957.

Development of Major Respiratory Allergies.—In the 100 children studied, major respiratory allergies developed as follows:

(1) Asthma	32
(2) Nasal allergies (includes perennial allergic rhinitis, pollinosis, and the recurrent upper respiratory disorder)	17
	49

(Those asthma patients who also had perennial allergic rhinitis, recurrent upper respiratory disorder, or pollinosis were not included in the second group.)

In most of the patients tabulated in the first group, the diagnosis of asthma had been made between one and one-half and two and one-half years after their original admission with acute bronchiolitis. In only two patients could a history of atopic dermatitis be elicited. There were three cases of repeated admission with acute bronchiolitis.

Family History of Major Allergies.—In the 100 cases studied, a reasonably reliable family history was available in only eighty-three.

Of these eighty-three families, thirty-seven (44 per cent) showed incidence of either asthma pollinosis, or eczema. The literature reviewed by Bray[3] indicates an incidence of not over 10 per cent in the families of normal persons.

The incidences of perennial allergic rhinitis and recurrent upper respiratory disorder were purposely omitted from this study of family history because of the laity's unfamiliarity with these terms and because of the relative vagueness of these conditions if history alone is used for their determination.

DISCUSSION

In spite of its synonym of "acute asthmatic bronchitis," its "certain clinical similarities with asthma,"[7] and its therapeutic response to certain types of medications often used in the treatment of asthma (such as aminophylline and ACTH),[4-6] bronchiolitis has rarely been considered an allergic process. It is generally believed to be an infectious disease, limited to a certain anatomic region and to a particular age group. Genuine asthma is usually mentioned as a differential diagnostic consideration[6] along with laryngotracheobronchitis, foreign bodies, cystic fibrosis of the pancreas, and others as noted by Nelson and Smith.[8]

According to Alexander,[2] histopathologic lesions of bronchial asthma embrace "edema, smooth muscle spasm, and increased secretions from mucus glands." All of these contribute to narrowing of the airways, particularly at the level of bronchioles with a diameter of 2 to 8 mm. Air is trapped in the alveoli, which distend to produce acute emphysema.

In bronchiolitis, a spastic element is not usually suspected, in spite of its occasional dramatic response to aminophylline and even to epinephrine. Nor do we see here the abundant mucous secretions which are found in the bronchiolar lumen in asthma. Finally, bronchiolitis is usually a one-time illness, lasting about one week, while recurrent paroxysms of short duration are characteristic of bronchial asthma. Unfortunately, detailed studies of eosinophilia in the nasal and bronchial mucus have not been reported, and in the few cases that come to necropsy a lymphocytic infiltrate of the small bronchi and bronchioles is noted with a mild to moderate infiltrate of neutrophils in the bronchiolar lumina and often in the adjoining alveolar spaces.[6]

With all these differentiating points in mind, there is at least a suggestion of a relationship between asthma and bronchiolitis, although the conditions discussed are certainly not identical. Our follow-up studies have revealed a 32 per cent incidence of asthma eventually occurring in children who have suffered from bronchiolitis. This seems to be more than accidental. One may well wonder if a single episode of bronchiolitis might prime the tissue structures of the involved areas for the development of later asthmatic disease, in the sense of a locus minoris resistentiae. Or could the local edema found in bronchiolitis constitute something like a forme fruste of the pathology usually found in asthma of later age groups? The high familial incidence of asthma in this study seems to suggest the latter possibility to some extent.

Whatever the answers to these questions may be, if our findings should be corroborated by other studies, then one would certainly be justified in observing a child with a history of acute bronchiolitis for the later development of respiratory allergy and in using all prophylactic measures available in the management of these children.[5]

SUMMARY AND CONCLUSIONS

(1) Acute bronchiolitis of infancy is usually considered as an infectious disease. A variety of microorganisms are suggested as possible etiological agents, *Hemophilus influenzae, Diplococcus pneumoniae,* and certain viruses being the most frequently suspected. Some authors favor a symbiotic combination of *H. influenzae* and a virus.

(2) We studied 100 children who had been admitted to the pediatric services of Rochester, New York, between 1950 and 1957 with a diagnosis of acute bronchiolitis. Most of these were infants. The purpose of this study was to ascertain how many of these children subsequently developed either bronchial asthma or other respiratory allergies and how many had a positive family history for allergy.

(3) Studies of hospital records and communication with the patients' families and with their private physicians indicated that thirty-two of 100 such children now have bronchial asthma, while an additional seventeen are suffering from either recurrent upper respiratory disorders, perennial allergic rhinitis, or pollinosis.

(4) The question is raised as to whether this high incidence of respiratory allergies (49 per cent) in children with a history of bronchiolitis could be of significance for an etiological relationship between bronchiolitis and asthma.

(5) A plea is made for further studies of a similar nature in other parts of the country. Meanwhile, it seems fair to assume that acute bronchiolitis in infancy should be looked upon as a condition which is often followed by the development of respiratory allergic disease, particularly asthma, and appropriate prophylactic measures should be instituted.

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ALLERGENIC EXTRACTS—A COMPARISON OF THEIR QUALITY AND RELIABILITY

Description of Technique for Preparation of Stabile Allergens and Proposal for Biologic Assay

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ALLERGENS and their application in the diagnosis and treatment of a variety of diseases have played a major role in the development of the specialty of clinical allergy. Yet, in recent years the skin test has lost much of its stature as an aid in allergy studies. Many physicians leave such studies entirely to technical assistants, who often are inexperienced in their application and interpretation. It is little wonder, therefore, that much of the medical profession is cynical about the value of skin testing, about specific allergy management based on such tests, and even about the specialty of allergy itself.

The value of skin testing as an aid to clinical allergy must depend on certain basic points:

1. Reliability of the allergen.
2. Method of application and interpretation of the reaction.
3. Incorporation of the results in over-all evaluation of the patient.

The second and third points were critically evaluated and offered by Tuft[1] fifteen years ago.

The present discussion will therefore be concerned only with quality and reliability of the allergens. Since most of the standard textbooks on allergy contain discussions of common methods of obtaining, handling, preparing, and standardizing extracts, there is no need for a detailed review of each allergen in common use. Certain basic general principles, however, will be outlined.

RAW MATERIAL

The source of raw materials, their handling, and their preservation represent the paramount preliminary steps in providing the final reliable allergens. Certain essential criteria must be fulfilled:

1. *The source of raw material should offer sufficient supply to permit the use of a reliable method for the concentration of an adequate quantity of the final allergenic product.* House dust, which is readily available in large quantities, permits elaborate methods for preparation of a highly concentrated and highly allergenic product.[2] On the other hand, many epidermals, which can be gathered only in limited quantity and which yield small amounts of active allergens, are often so inadequately concentrated as to provide testing materials with questionable allergenicity.

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2. *The raw material should be obtained and handled in a manner that will prevent the deterioration of the intrinsic allergenic factor.* In the case of many fruits and vegetables and some cereals, pigmentary oxidation may occur within a few hours.[3] Epidermals, such as feathers and hog hair, are seriously affected in the course of removal with scalding hot water. Meats, fish, and insect material require rapid processing before autolytic changes occur.

3. *The raw material should be uncontaminated by other allergic substances.* This may necessitate acquaintance with the source of the raw material and with the manner and care of its handling before the extract is made. During storage many epidermals may be contaminated with house dust. On the other hand, house dust from an unknown source may be contaminated with the epidermals of household pets.

4. *Pollens, if obtained in original dry form, should be inspected grossly and microscopically to see that the following requirements have been met:*

(a) The pollen under the microscope is the same as that indicated on the label. (Errors in labeling do occur.)

(b) There are no other contaminating pollens present.

(c) The pollen is normal in appearance and does not show deterioration from age or moisture.

(d) There is no appreciable amount of moisture or debris. This is especially important when weight/volume is the method used for preparation of the pollen extract.

5. *Gathering of insects in quantity may require many hands and a general knowledge of entomology.*[4] Some insects, especially those which swarm, can be readily caught in large numbers. A few insects, such as cockroach, mosquito, and other dipterae, can be raised in quantity in the laboratory.

Preservation of insects by quick freezing from the live stage will prove most successful. By this method, mildewing and putrefaction are avoided. Defatting, lyophilizing, and fine grinding in a Wiley mill will yield a product which may be stored indefinitely under desiccation until needed in the preparation of aqueous extracts. Some insects, such as moth, caddis fly, mosquito, and a few others, can be simply air dried, defatted, and then kept under desiccation without the more elaborate procedure used for the larger-bodied insects.

PREPARATION OF ALLERGENS

The ideal method for the preparation of allergenic extracts will fulfill certain rigid postulates:

1. The method will yield a final extract which is actively allergenic.

2. The method will yield a final extract which is free of nonspecific irritants.

3. The method will yield a final extract which is stabile for many months or years.

4. The method will not be too complex or costly.

Unfortunately, no present method fulfills ideally such rigid postulates for the preparation of all allergens. The ideal method must adapt itself to the nature of the raw material and to the allergen being prepared.

Too frequently the compromise sacrifices reliability for the sake of simplicity and economy. Such shortsightedness yields an unreliable allergen which offers only costly misinformation to the physician and his patient.

Proposed Method of Preparing Most Allergens in Quantity.—In our laboratory there has been in use for several decades a method which has provided reliable and stabile allergens free from nonspecific irritants. This method, once established, is not cumbersome and not too costly in light of the quality of the final allergen.

Briefly, this method consists of adding four parts acetone to a clear, crude, buffered aqueous extract. The resultant flocculant precipitate is allowed to settle to the bottom over a period of hours, and the supernatant liquid is poured off. The precipitate, with a small amount of residual liquid, is poured into a filter funnel and, after filtration, is washed with isopropyl alcohol (99 per cent pure) and finally with ethyl ether. This final precipitate is placed in a desiccator for several days, at the end of which time it is thoroughly dried and readily pulverized. In the case of slow-drying allergens, lyophilization has proved more satisfactory.

A most important aspect of this procedure is the carrying-out of all steps under subfreezing temperature (about 28° to 30° F.). The final desiccated extract is finely ground in a Wiley mill, using an 80-mesh screen. This fine powder is then stored indefinitely under desiccation.

Before it is accepted for storage (and for future routine use), the dry powdered material is assayed by scratch testing on known sensitive patients. Only if it reacts as strongly as the allergen currently in use and, at the same time, does not cause any nonspecific irritation in nonsensitive persons, is it added to the common pool. Since only a few grams of such an allergen, freshly prepared and proved strongly reactive by skin testing, are added to their kind, there is maintained a reasonably constant quality which does not need repeated standardization chemically or immunologically.

For later clinical use, the dry powder is redissolved in a 50 per cent buffered glycerinated extracting fluid and passed through a sterilizing Seitz filter. The strength of dilution varies from 1:25 to 1:50 by weight/volume, depending upon the solubility of the powdered material. (This has been established through experience.) The allergen in solution is then ready for further dilution in testing and treatment of the patient.

This method of preparing powdered concentrate has been applied to epidermals, dusts, foods, and many miscellaneous allergens that require concentration. Although zinc sulfate, ammonium sulfate (saturated and half-saturated strength), mercuric chloride, and other precipitating salts were used experimentally in the past, especially for fractionation of various possible allergenic components, they required the employment of cumbersome equipment and lengthy methods that acetone precipitation has now eliminated.

This method of precipitating out the active allergen with a water-miscible solvent, sketchily outlined, omits some inevitable pitfalls and disappointments. Certain epidermals, such as those of the rabbit and the cat, yield a niggardly amount of precipitates in proportion to the effort and the raw material used. A number of precipitates (especially orris root) dry very slowly unless lyophilized; others are slow to redissolve. We have a "dossier" on each allergen prepared in our laboratory, which spares us the repetition of frustrating and disappointing experiences. With this method, epidermals and miscellaneous inhalants have provided excellent allergens that are uniformly reliable and have remained stabile indefinitely in the dry form. Most of the foods have also been prepared by acetone precipitation and have provided actively allergenic material that is ready for use at any season of the year, thus eliminating the necessity of a hurried visitation to the market place for such items as fresh fruits or vegetables.

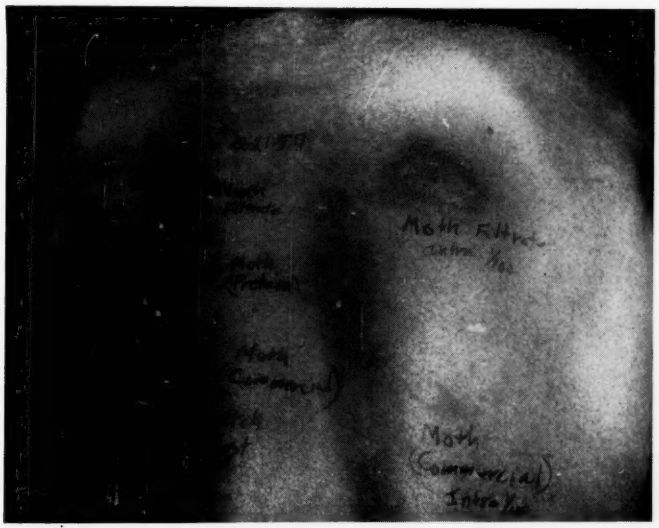

Fig. 1.—Moth extracts. Patient D. N., 44-year-old tire shop owner, had chronic asthma and extensive eczema since early youth. Skin testing showed moderate reactions to epidermals and house dust with strongest reactions to mold and insect extracts.

Comparison using moth filtrate (1:10 w/v) prepared from carefully handled material and "moth protein" prepared by acetone precipitation showed strong reaction by scratch testing. Intradermal test with the moth filtrate 1:100 gave a large reaction. Powdered moth from commercial source, in same strength, gave no reaction to the scratch test and an equivocal reaction on intradermal testing.

Pollens and Insects.—Pollens in the dry state and insects after lyophilization are defatted and then extracted for forty-eight hours at room temperature in 50 per cent buffered glycerin. In the case of pollens, a 1:50 by weight volume has been accepted as a standard. In the case of insects, the powdered material contains more inert material and a dilution of 1:10 by weight volume is the standard used. With pollens, little or no evident loss of allergenicity occurs throughout the entire season.[5] Some insect extracts have retained high protein-nitrogen content and still have shown strong skin reactions after more than a year.[4]

Both orchard grass pollens and several insects (moth, honeybee, and mosquito) were highly concentrated by acetone precipitation. These, as we expected, yielded a high protein-nitrogen content and showed very strong skin reactions even in great dilution. At this time, however, there seems to be no practical advantage in using this method of concentration, since both pollens and insects can be stored indefinitely in a dry, defatted, powdered state. Nonspecific reactions are more frequent when simple extracts are used, but these are represented chiefly by delayed "tuberculin type" reactions and are not clinically important. These nonspecific reactions are more frequent for insect extracts than for the pollens.

COMPARATIVE STUDY OF QUALITY OF ALLERGENS

For purposes of comparison of the quality of allergens currently in use, testing material was obtained from seven sources representing five commercial

Fig. 2.

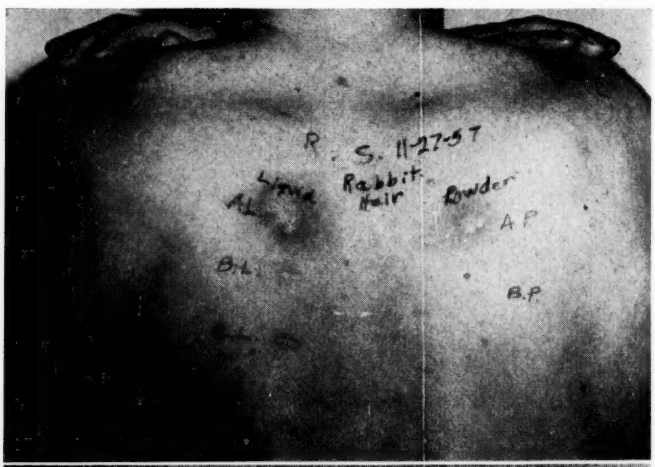

Fig. 3.

Fig. 2.—Rabbit epidermal. Patient R. S., 17-year-old son of a farmer. Perennial allergic rhinitis was present for several years with severe symptoms during the pollen season. The patient reacted strongly to pollens and epidermals.

Comparison, in this case, showed positive skin reactions only with "A.L." and "A.P." This contrast of reaction on several known sensitive patients was repeatedly demonstrated.

Fig. 3.—Goat epidermal. Patient E. M., 29-year-old rancher had asthmatic attacks and chronic perennial allergic rhinitis dating back to childhood. He strongly suspected epidermals and hay, and while in military service in Japan he was free of allergic symptoms. Most of the epidermals tested by the scratch method showed strongly positive reactions.

Comparison, using powder and liquid, showed that material "A.L." and "A.P." reacted strongly. The material from other sources failed to show any reactions.

laboratories and two allergy clinics. The commercial laboratories were widely distributed throughout the United States and would no doubt comprise the source of allergens currently used for skin testing by most physicians who do not prepare their own extracts.

For obvious reasons, the sources of supply of the various allergens are designated in the illustrations only by the letters "A" through "F." After each of these letters there is a designated letter, "P," for powdered material, and "L," for liquid material. "A" is revealed as the allergen from our laboratory, prepared by acetone precipitation. The liquid for allergen "A" is prepared from the dried powder by dissolving in 50 per cent buffered glycerin in a concentration of 1:50 by weight volume. No detailed information was available as to the method used in preparation of materials obtained from other sources. The allergens in powdered form were obtained from only two other laboratories. In the case of several epidermals, this material had the appearance, on close inspection, of finely ground hair. One commercial laboratory complied with our request for special preparation of an acetone precipitate of cat and dog epidermals. Although the quantity sent to us was too small for chemical analysis, it may be noted in the subsequent illustrations that the skin reactions were strong.

Patients who gave histories of hay fever or asthma and who even suspected specific epidermals were chosen. In the case of sensitivity to foods, the patient was certain of their offending quality. None of the patients represented in the illustrations (Figs. 1 to 10) had in recent years received specific desensitization or were currently on antihistaminics or other symptomatic medication. The illustrations reveal the unreliability of many of the allergens applied by the scratch method.

DISCUSSION

Although investigative studies in allergy and applied immunology are carrying current thought onto somewhat remote paths, it is still important to re-examine the basic clinical tools used daily in the study and care of the allergic patient. Figs. 1 to 10 demonstrate consistent findings of the unreliability of the allergens studied, which represent many of those allergens widely used. The epidermals chosen are used frequently in routine skin testing and are important in diagnosis and specific desensitization.

All the allergens were obtained (shortly before starting these studies) from commercial sources or from allergy clinics where they were in current use. Most of the vials showed some symbols of standardization, either as to nitrogen content or weight/volume dilution. Some labels also indicated the type of diluent used, the date of preparation, and the date of expiration. None of the labels indicated that positive skin reactions had been obtained with the contained allergen. We made no attempt to gain detailed information concerning the method of preparing these allergens. The illustrations, however, give evidence of consistent findings that only allergen samples "A" and several labeled "C" and "F," prepared by acetone precipitation, gave consistently strong, positive skin reactions.

Our method of preparing a concentrated allergen in stabile powder form by acetone precipitation is not a recent development. It has been the standard method for preparation of epidermals, foods, and most miscellaneous inhalants (except pollens and insects) in our laboratory for more than twenty years. It has been employed even longer by a few other allergists.

The preparation of purified house dust extract, using such a water-miscible organic liquid as that described by Boatner and associates,[2, 6] includes

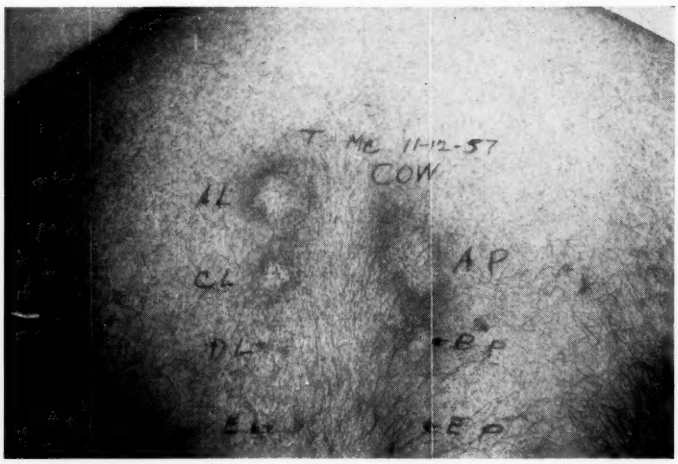

Fig. 4.—Cow epidermal. Patient T. McC., 56-year-old physician who raised registered beef cattle developed asthma each time he groomed cattle for stock show. Strongest reactions were to cow epidermal, and there were moderate reactions to hay, house dust, and several other epidermals.

Comparison shows strong positive scratches to samples "A.L." and "A.P." with moderate reactions to "C.L." and "B.P.," and no reaction to those from other sources.

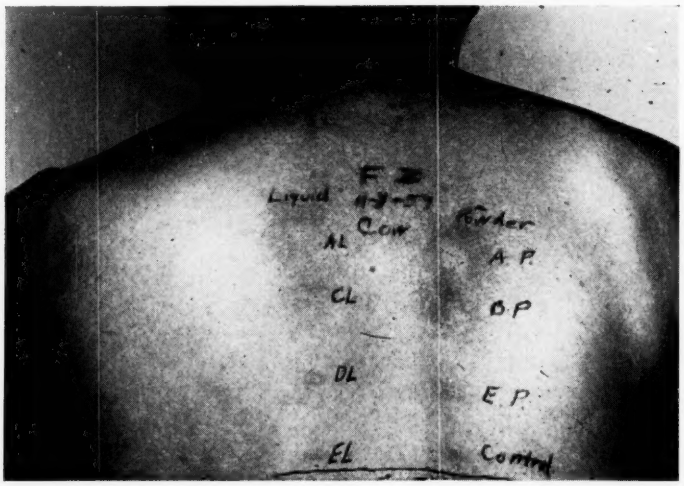

Fig. 5.—Cow epidermal. Patient F. Z., 47-year-old farmer. Asthmatic attacks are continual but most intense after contact with farm animals and hay. Strong positive skin reaction by scratch testing with epidermals and pollens.

Comparison shows sample "A.P." and liquid "A.L." producing strong whealing reactions.

The powder "B.P." and liquid "C.L." from commercial sources reacted moderately. The remaining samples showed no reactions.

the preliminary step of fractional precipitation first with a "low concentration" of an organic liquid using two parts dioxine to three parts crude aqueous extract, thus removing the inert fraction. The active allergen is precipitated out with the "intermediate concentration." Further purification was done with ammonium sulfate. The high allergenic quality of house dust prepared by the method of Boatner and colleagues and sold as Endo house

Fig. 6.—Dog epidermal. Patient N. S., 35-year-old paper maker. Perennial allergic rhinitis and mild asthma date back four years. He had no household pets at this time but showed, on testing, strong reactions to epidermals and house dust as well as to grain cereals.

Comparison, using materials "A.L." and "A.P.," showed consistently strong scratch reactions, while the others failed repeatedly to show any significantly positive reaction.

Fig. 7.—Dog epidermal. Patient C. F. V., 20-year-old construction worker. Recurrent eczema and minimal hay fever date back to childhood. Testing produced strong skin reactions to epidermals, pollens, and insect extracts.

Comparison showed reactions only with the liquid extract "A.L." and with the powdered allergens "A.P." and "C.P." and lyophilized saliva. The sample "C.P." was especially prepared by a commercial laboratory using the method of acetone precipitation (author's request). The other samples of dog epidermal failed to react.

dust has been well established, as evidenced by its wide use among many allergists. Their method of fractional precipitation is adaptable to house dust, since the crude material is available in limitless quantity and yields a relatively large amount of intrinsic allergenic material. Such epidermals as those

Fig. 8.

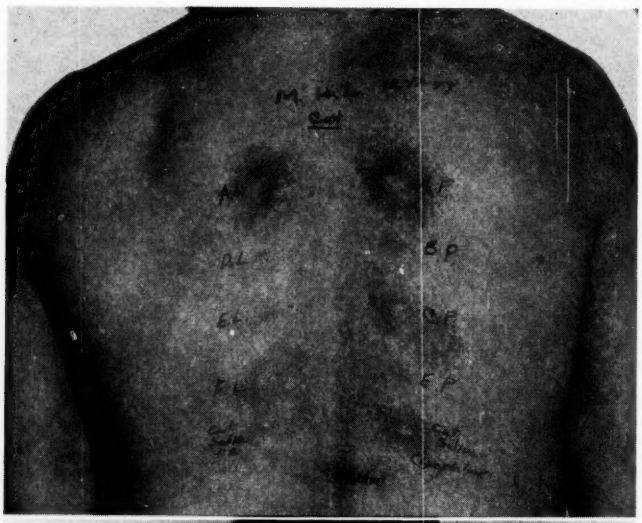

Fig. 9.

Fig. 8.—Cat epidermal. Patient M. H. L., 34-year-old mechanic. Allergic rhinitis and mild asthma dated back to childhood. Strongest skin reactions were obtained with epidermals and house dust.

Comparison showed that cat epidermal reaction was strongest to the prepared allergen "A.L." and "F.L." The powdered allergen "A.P." and "C.P." and lyophilized cat saliva also showed strong reactions. ("F.L." and "C.P." were from the same commercial source.)

Fig. 9.—Cat epidermal. Patient L. G., 29-year-old grocery clerk, developed asthma one year previous to being tested. He reacted strongly to cat epidermal and moderately to other inhalant allergens.

Comparison of concentrated cat epidermal showed the powder form "A.P." and "C.P." reacted strongly. Also, the powder prepared by simply lyophilizing cat saliva (obtained from an anesthetized animal stimulated with pilocarpine to promote salivation) gave a significantly positive skin reaction. The liquids "A.L." and "B.L." and the original cat saliva, simply diluted with equal parts of buffered solution, produced a strong reaction. All other materials applied failed to show any skin reactions.

of the cow, the dog, and the horse would also lend themselves to this method of fractional precipitation, since they too are available in large quantities and yield a high quantity of intrinsic allergens.

A few epidermals, on the other hand, especially those of the cat and the rabbit, provide with a single-step precipitation (as previously described in our own method) a small quantity of highly soluble, strongly allergenic powder which redissolves completely and almost instantaneously. The preliminary step with low-concentration acetone and later use of ammonium sulfate, as used in the preparation of Endo house dust, would not be necessary.

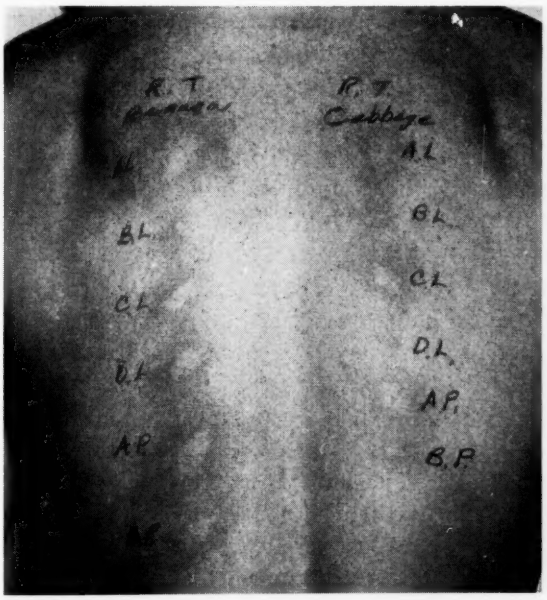

Fig. 10.—Food allergens. Patient R. T., 21-year-old student. Asthma and perennial allergic rhinitis date back to age 4. He was aware that several foods, especially banana, caused gastrointestinal symptoms. Skin testing revealed strong reactions to epidermals, pollens, and many foods.

Comparison of extracts of banana and of cabbage, from various sources, showed lack of allergenicity of several samples. With banana, only the liquid "B.L." failed to cause skin reaction. With cabbage, "B.L." (same commercial source) and "D.L." failed to elicit skin reactions.

It is evident that, for the sake of economy and simplicity, even the method of Boatner and associates for preparing a pure allergen by precipitating the active ingredient using a water-miscible organic liquid, such as acetone, must be adapted to the raw material used. The final allergenic extract will almost inevitably prove satisfactory for clinical use. The dry, powdered material, when proved allergenic, will also be found free from nonspecific irritating substance and can be stored indefinitely under desiccation. Redissolving this material in buffered 50 per cent glycerin, using a standardized method, should on each occasion yield a standard extract which is stabile for many months. For those who employ biochemical analyses as presumptive evidence of strength of an allergen, the total-nitrogen and protein-nitrogen studies can be done to confirm the constant quality and stability of these allergens.

CONCLUSIONS

1. The allergenic extracts in common use, obtained in both powdered and liquid forms from various commercial sources, have frequently proved biologically inert, as evidenced by negative skin reactions in known sensitive patients.

2. The use of water-miscible organic liquid, particularly acetone, in the preparation of concentrated extracts of house dust as well as other allergens has proved highly satisfactory. If this method is applied on a larger commercial scale and modified for various allergens, it can provide a method that would not be costly and would not be too cumbersome. It could provide uniform material to all physicians and thus lead to a common language in our discussion of clinical allergy.

3. Since skin testing and desensitizing with specific allergens are important procedures in the practice of allergy, materials of known worth should be made available to those who will employ them.

4. The present method of standardization on the basis of chemical content requires skilled hands and, at best, it still fails to prove specific biologic activity of the allergen.

5. Although no method for standardizing allergens has been universally accepted, the physician should at least be given some assurance that the material has been biologically assayed in that it has strong whealing activity and contains no significant amount of nonspecific irritating substance.

6. The national societies of allergy, together with the regional and local allergy groups, might well accept the responsibility for assaying allergens biologically. In the formative period this may involve the need for making available allergens of known quality to be used in comparative studies with newly prepared material. The commercial laboratories engaged in the preparation of allergens should certainly welcome this. Those clinicians depending on allergens from these commercial sources will, as a result, feel more secure in what they are employing in their daily practice.

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COMPARATIVE EFFECTIVENESS OF AN ALCOHOL-WATER SOLUTION OF THEOPHYLLINE (ELIXOPHYLLIN), ALCOHOL-WATER SOLUTION, AND THEOPHYLLINE-WATER SOLUTION FOR THE ORAL TREATMENT OF ACUTE BRONCHIAL ASTHMA

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THE literature amply attests to the effectiveness of theophylline in relieving bronchial asthma.[1, 2] For years intravenous aminophylline, a theophylline compound, has been employed to treat acute asthmatic attacks. The intravenous route of administration has been found highly efficacious, but its use requires a physician's services and occasionally severe side reactions are noted. Rapid response to intravenous aminophylline, in contrast to the ineffective response to aminophylline administered orally, strongly suggests that the relief obtained may be dependent upon the height of the resulting blood theophylline level and the rapidity with which it is reached.[3, 4]

Waxler and Schack[5] showed that the mean theophylline blood levels obtained with oral aminophylline in tolerated doses and with aminophylline suppositories one-half hour following their administration were far below effective levels. When a large oral dose of aminophylline is given, the resulting xanthine in the blood is similarly inadequate,[6] and severe gastric irritation frequently follows the administration of the larger oral doses of this compound.[7]

Schluger, McGinn, and Hennessy,[6] investigating a new theophylline preparation, Elixophyllin,† reported mean blood theophylline levels of 8.0 mcg. and 10.3 mcg. per milliliter of blood fifteen and thirty minutes, respectively, following the oral administration of a single dose of 5 tablespoonfuls of Elixophyllin, equivalent to 0.40 gram of theophylline. These values exceeded those obtained by Bradwall,[8] 7 mcg. at fifteen minutes, and by Waxler and Schack,[5] 6 mcg. one-half hour after the intravenous injection of 0.30 gram and 0.25 gram of aminophylline, respectively.

In a previous study[7] Elixophyllin was found to be of value in the oral treatment of acute and chronic asthma. A single dose of 5 tablespoonfuls (75 ml.) promptly terminated severe acute asthmatic attacks. Repeated daily doses of this drug proved more effective than comparable doses of aminophylline in mild, moderately severe, and severe chronic asthma with persistent wheezing. In this previous study the therapeutic effectiveness of Elixophyllin was ascribed

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†Elixophyllin is a product of Sherman Laboratories, Detroit, Michigan.

chiefly to faster and more efficient absorption of theophylline from the gastrointestinal tract. Distressing gastrointestinal effects did not occur with the high doses of the new theophylline preparation used in this study.

The usefulness of Elixophyllin has also been reported by Schluger, McGinn, and Burbank,[9] Kessler,[10] Greenbaum,[11] and Slepian.[12] These investigators reported excellent to good responses to Elixophyllin in the treatment of asthma. These studies emphasize the fact that this is a theophylline preparation which can be administered orally and yet give about the same results as intravenously administered aminophylline. Moreover, they demonstrated also that patients prone to sudden acute attacks of asthma could treat themselves at home, thus avoiding the necessity for emergency visits by the physician or trips to a hospital emergency room. The intravenous administration of aminophylline and the dangers incident thereto could thus be eliminated.

PHARMACOLOGY

Elixophyllin is a hydroalcoholic solution containing 80 mg. of free theophylline and 3 ml. of ethyl alcohol per tablespoonful (15 ml.). Each tablespoonful contains the theophylline equivalent of 100 mg. of aminophylline. Since the theophylline present in Elixophyllin is in the free form, more rapid and efficient absorption is expected to occur than from tablets of theophylline salts. Unlike aminophylline and similar basic salts of theophylline, the theophylline present in this hydroalcoholic solution is not precipitated onto the gastrointestinal mucosa by the action of hydrochloric acid.

The present study may be considered an extension of the previous investigation. It deals primarily with the attempt to obtain a more direct answer to the question: "To what attribute does this oral theophylline preparation owe its unique properties?"

Since Elixophyllin is a hydroalcoholic theophylline solution, it is to be expected that the enhanced therapeutic effectiveness observed following its oral use has to be ascribed to one of the actions listed:

1. The single action of the alcohol or the free theophylline present in Elixophyllin.
2. The sum of the two single actions.
3. The potentiation of activity or synergism.
4. Speeded and more efficient absorption of theophylline.

To determine which of the actions is responsible for the effectiveness of Elixophyllin, it was decided to test appropriate alcohol and theophylline control solutions alone and in combination. The results obtained with the use of control preparations were compared with those recorded after the use of Elixophyllin.

MATERIALS AND METHODS

Altogether, three products were evaluated. The first was a hydroalcoholic control solution containing the equivalent of 3 c.c. of ethyl alcohol and approximately 12 c.c. of water per tablespoonful. The other control product had no

alcohol but did contain 80 mg. of theophylline and approximately 15 c.c. of water per tablespoonful. Flavoring and coloring materials were added to both control preparations in order that those solutions might match as closely as possible the taste and color of Elixophyllin, the third product being evaluated.

The procedural design employed in this study was similar to the one that I used previously[7] to evaluate the efficacy of a single oral dose of five tablespoonfuls (75 c.c.) of Elixophyllin in the treatment of severe acute asthmatic attacks. Briefly, the patients who presented themselves for treatment during an acute asthmatic attack were given a single dose of one of the control preparations and a combination of them in sequence.

Prior to and at five, fifteen, and thirty minutes following the administration of the medications, vital capacity measurements were performed. Results were judged on the basis of subjective and objective clinical improvement, together with changes in vital capacity.

RESULTS

The results in fifty patients are analyzed. They include the results in the ten patients who received the alcohol control, the ten who received the theophylline control, the twenty who were given the alcohol-theophylline preparation, and the ten who were pretreated for a period of fifteen minutes with the alcohol solution and then received the theophylline solution.

TABLE I. INDIVIDUAL RESULTS OF TREATMENT OF SEVERE ACUTE ASTHMATIC ATTACK WITH SINGLE ORAL DOSE OF 5 TABLESPOONFULS (75 ML.) OF CONTROL ALCOHOL PREPARATION*

		VITAL CAPACITY MEASUREMENTS			
			AFTER Rx		
CASE NO.	CLINICAL RESPONSE	BEFORE Rx (C.C.)	5 MINUTES (C.C.)	15 MINUTES (C.C.)	30 MINUTES (C.C.)
1A	None	1,100	1,100	1,200	1,250
2A	None	1,500	1,500	1,500	1,500
3A	None	2,000	2,100	2,100	2,200
4A	None	2,000	2,100	2,100	2,150
5A	None	1,800	1,950	2,000	2,000
6A	None	2,400	2,300	2,250	2,450
7A	None	1,900	2,000	2,100	2,150
8A	None	1,750	1,800	1,800	1,850
9A	None	2,200	2,400	2,500	2,500
10A	None	1,800	1,800	1,850	1,900

*This control preparation is a 20 per cent hydroalcoholic solution in which each tablespoonful (15 c.c.) contains the equivalent of 3 c.c. of ethyl alcohol and the remainder consists of water (approximately 12 c.c.). Small amounts of flavoring and coloring materials have been added so as to match as closely as possible the taste and color of Elixophyllin.

Tables I and II represent the individual responses obtained following the administration of a single oral dose of 5 tablespoonfuls (75 c.c.) of the alcohol control and of the theophylline control. None of the ten patients treated with the hydroalcohol solution showed any clinical improvement, while two of the ten placed on the theophylline-aqueous solution exhibited a good clinical response and the remaining eight showed questionable or no improvement.

TABLE II. INDIVIDUAL RESULTS OF TREATMENT OF SEVERE ACUTE ASTHMATIC ATTACK WITH SINGLE ORAL DOSE OF 5 TABLESPOONFULS (75 ML.) OF CONTROL THEOPHILLINE PREPARATION*

CASE NO.	CLINICAL RESPONSE	VITAL CAPACITY MEASUREMENTS			
			AFTER RX		
		BEFORE RX (C.C.)	5 MINUTES (C.C.)	15 MINUTES (C.C.)	30 MINUTES (C.C.)
1B	Equivocal	2,000	2,100	2,200	2,400
2B	Equivocal	2,000	2,200	2,400	2,400
3B	Equivocal	1,100	1,300	1,300	1,500
4B	Equivocal	1,100	1,250	1,300	1,300
5B	Equivocal	1,500	1,500	1,600	1,750
6B	Good	1,800	1,800	2,100	2,350
7B	Equivocal	2,200	2,150	2,350	2,600
8B	Equivocal	1,000	1,100	1,150	1,400
9B	Equivocal	2,100	2,100	2,150	2,350
10B	Good	1,700	1,850	2,000	2,300

*This control preparation is a hydrotheophylline solution in which each tablespoonful (15 c.c.) contains 80 mg. of free theophylline and the remainder consists of water (approximately 15 c.c.). Small amounts of flavoring and coloring have been added so as to match as closely as possible the taste and color of Elixophyllin.

Average increases in vital capacity at the end of thirty minutes were 150 c.c. after the alcohol control and 295 c.c. after the theophylline control. The sum of the average increases after the use of the two control preparations amounted to 445 c.c. at thirty minutes.

Table III represents a summary of the individual responses occurring after a single oral dose (75 ml.) of Elixophyllin. Of the twenty patients treated,

TABLE III. INDIVIDUAL RESULTS OF TREATMENT OF SEVERE ACUTE ASTHMATIC ATTACK WITH SINGLE ORAL DOSE OF 5 TABLESPOONFULS (75 ML.) OF ELIXOPHYLLIN*

CASE NO.	CLINICAL RESPONSE	VITAL CAPACITY MEASUREMENTS			
			AFTER RX		
		BEFORE RX (C.C.)	5 MINUTES (C.C.)	15 MINUTES (C.C.)	30 MINUTES (C.C.)
1	Excellent	2,400	2,700	2,800	3,200
2	Excellent	1,700	2,100	2,200	2,400
3	Excellent	2,200	2,700	3,000	3,400
4	Good	1,500	1,700	2,000	2,100
5	Excellent	2,400	2,800	2,900	3,200
6	Excellent	2,250	2,500	2,700	3,000
7	Good	1,900	2,050	2,300	2,450
8	Excellent	2,100	2,500	2,800	3,200
9	Good	1,700	1,850	2,100	2,200
10	Good	2,300	2,550	2,700	2,850
11	Excellent	1,950	2,400	2,800	3,000
12	Excellent	2,400	2,700	2,950	3,300
13	Excellent	1,700	2,000	2,400	2,650
14	Excellent	2,300	2,500	2,800	3,200
15	Excellent	1,800	2,100	2,500	2,700
16	Good	2,500	2,600	2,900	3,150
17	Excellent	2,350	2,800	3,000	3,250
18	Excellent	2,100	2,650	3,000	3,050
19	Good	1,800	1,950	2,200	2,300
20	Excellent	2,400	2,600	3,000	3,300

*Elixophyllin is a hydroalcoholic theophylline solution in which each tablespoonful contains the equivalent of 3 c.c. of ethyl alcohol and 80 mg. of free theophylline. The remainder consists of water (approximately 12 c.c. per tablespoonful) and small amounts of coloring and flavoring materials.

fourteen obtained excellent relief and six experienced good relief. Clinical improvements observed were accompanied by significant increases in vital capacity measurements. The average increase in vital capacity was 809 c.c. at thirty minutes.

Individual results of treatment of severe acute asthma following the administration of a single oral dose of 5 tablespoonfuls of the theophylline control after a fifteen-minute period of pretreatment with a similar quantity of the alcohol control are shown in Table IV. None was improved by the alcohol control alone, but all obtained excellent response after the subsequent administration of the theophylline control. The average increases in vital capacity were 600 c.c. and 1,090 c.c. thirty and forty-five minutes, respectively, after start of treatment.

TABLE IV. INDIVIDUAL RESULTS OF TREATMENT OF SEVERE ACUTE ASTHMA ATTACK WITH SINGLE ORAL DOSE OF 5 TABLESPOONFULS (75 ML.) OF CONTROL THEOPHYLLINE PREPARATION AFTER A FIFTEEN-MINUTE PERIOD OF PRETREATMENT WITH A SINGLE ORAL DOSE OF 5 TABLESPOONFULS (75 ML.) OF CONTROL ALCOHOL PREPARATION

	CLINICAL RESPONSE		VITAL CAPACITY MEASUREMENTS				
				AFTER ALCOHOL CONTROL		AFTER THEOPHYLLINE CONTROL	
CASE NO.	AFTER ALCOHOL CONTROL	AFTER THEOPHYLLINE CONTROL	BEFORE Rx (C.C.)	5 MINUTES (C.C.)	15 MINUTES (C.C.)	15 MINUTES (C.C.)	30 MINUTES (C.C.)
1C	None	Excellent	1,100	1,100	1,200	2,000	2,400
2C	None	Excellent	1,100	1,100	1,300	2,000	2,800
3C	None	Excellent	2,100	2,200	2,350	2,800	3,300
4C	None	Excellent	2,300	2,350	2,450	3,000	3,350
5C	None	Excellent	1,800	2,000	2,000	2,700	3,100
6C	None	Excellent	1,500	1,500	1,650	1,900	2,350
7C	None	Excellent	2,400	2,450	2,500	2,800	3,300
8C	None	Excellent	1,300	1,450	1,550	1,600	1,950
9C	None	Excellent	1,900	2,000	2,000	2,200	3,000
10C	None	Excellent	2,100	2,050	2,200	2,600	2,950

DISCUSSION

Examination of Table V reveals that the over-all effect elicited by Elixophyllin in terminating an acute asthmatic attack within thirty minutes cannot be ascribed to single actions of its alcoholic or theophylline content or to the sum of the single actions of both components.

This conclusion is based on a comparison of clinical responses observed and of increases in vital capacity measurements. All patients responded to treatment with Elixophyllin, but none responded to the alcohol control and only two to the theophylline control. Of the twenty patients treated with the two control preparations, only two showed therapeutic increases in vital capacity. However, the amount of such enhancement approached only the lowest individual increases observed following the administration of Elixophyllin.

This comparison clearly indicates that the "unexpected effects" noted with the use of Elixophyllin cannot be attributed to the single actions or to additive effects of the alcohol and theophylline.

Examination of results of treatment with a single dose of 5 tablespoonfuls of Elixophyllin and with the combination of the controls given in sequence (Table V) demonstrates that they are about equally effective in terminating an attack. Yet, neither the alcohol control nor the theophylline control administered alone produces any demonstrable clinical effects. Moreover, the sum of the average increases in vital capacity due to the alcohol control and the theophylline control is 445 c.c. after thirty minutes and 1,090 c.c. after the theophylline control following a fifteen-minute period of pretreatment with the alcohol control.

TABLE V. RESULTS OF TREATMENT OF SEVERE ACUTE ASTHMATIC ATTACKS WITH SINGLE ORAL DOSES OF 5 TABLESPOONFULS (75 ML.) OF (1) ALCOHOL CONTROL PREPARATION, (2) THEOPHYLLINE CONTROL PREPARATION, (3) ELIXOPHYLLIN, AND (4) ALCOHOL CONTROL PREPARATION AND THEOPHYLLINE CONTROL PREPARATION GIVEN IN SEQUENCE

DRUG AND DOSE	NO. OF PATIENTS	CLINICAL RESPONSE			MEAN VITAL CAPACITY MEASUREMENTS		
					BEFORE Rx: TOTAL (C.C.)	AFTER Rx: INCREASE	
		EXCELLENT	GOOD	EQUIVOCAL OF NONE		15 MINUTES (C.C.)	30 MINUTES (C.C.)
(1) Alcohol control, 5 tablespoonfuls	10	0	0	10	1,845	120	150
(2) Theophylline control, 5 tablespoonfuls	10	0	2	8	1,650	205	295
Arithmetic summation of Nos. 1 and 2	20	0	2	18	1,748*	325	445
(3) Elixophyllin, 5 tablespoonfuls	20	14	6	0	2,085	567	809
(4) 5 tablespoonfuls alcohol control followed in fifteen minutes by 5 tablespoonfuls theophylline control	10	10	0	0	1,760	160†	600‡
							1,090 (45 minutes)§

*Mean vital capacity measurements of twenty patients.
†Increase fifteen minutes after alcohol control (theophylline control given at this point).
‡Total increase in thirty minutes after start of therapy (fifteen minutes after theophylline control).
§Total increase in forty-five minutes after start of therapy (thirty minutes after theophylline control).

These comparisons demonstrate that the alcohol in Elixophyllin is needed to induce a therapeutic theophylline blood level within thirty minutes. Without the simultaneous use of alcohol or a short period of pretreatment with alcohol, the theophylline in Elixophyllin does not become available in optimal amounts to elicit prompt therapeutic benefits.

This explanation of the enhancement of the theophylline effect is supported by the findings of Schluger, McGinn, and Hennessy.[6] Blood theophylline levels of 10.3 mcg. per cubic centimeter of blood were observed after the administration of a single dose of 5 tablespoonfuls of Elixophyllin at thirty minutes.

With the theophylline equivalent dose of 0.5 gram of aminophylline, the blood theophylline level reached after thirty minutes was only 3.8 mcg. per cubic centimeter of blood.

SUMMARY

Asthmatic attacks were successfully terminated and vital capacity measurements were significantly increased thirty minutes after the oral administration of a single dose of 5 tablespoonfuls (75 ml.) of Elixophyllin. Results of control studies clearly indicate that the alcohol present in the preparation is responsible for the "unexpected effects" of theophylline administered orally.

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FOOD SENSITIVITY

A Study of 150 "Allergic" Children

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ALTHOUGH there is substantial literature on food allergy, there still exists some confusion as to its incidence and the extent of its relationship to allergic diseases. The purpose of this article is twofold—(1) to determine the incidence of food sensitivity in children with well-defined allergic disease and (2) to assess the role that food sensitivity plays in the various allergic disease entities. Numerous specific sensitivities to substances other than food were encountered in the children studied, but only food sensitivity is the object of this presentation.

CLINICAL MATERIAL

This series consists of 150 consecutive "allergic" children studied from 1955 through 1957. The group includes 119 private patients studied by one of us (S. S. F.) and thirty-one patients from the Rhode Island Hospital Pediatric Allergy Clinic studied by both of us. All but four or five of the referred children suffered from well-established allergic diseases, the duration of which ranged from four months to twelve years, with an average duration of two years and nine months at the time of the initial visit. Each child was referred for study and for advice on management (Table I).

CRITERIA FOR FOOD SENSITIVITY

As a screening test to enable us to isolate quickly all probable food-sensitive children, we accepted a positive skin test, a positive history, or both as preliminary evidence of food allergy. To obtain final evidence of food allergy, more rigid criteria were applied. We accepted either a strongly convincing clinical history of violent or repeated characteristic allergic symptoms, repeated positive test feedings, or both. Many times the history was so convincing and the child so ill that it was deemed unnecessary or even harmful to attempt provocative test feedings; in such cases test feedings were omitted.

Preliminary Evidence of Food Sensitivity in 150 "Allergic" Children.—Table II shows the number of children who gave a positive clinical history of food sensitivity and positive skin tests to foods in each allergic disease entity. Perennial asthma and atopic eczema are combined into one group. Seasonal asthma and hay fever are good contrasts and are less likely to show associated

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TABLE I. AGE AT INITIAL INTERVIEW OF 150 "ALLERGIC" CHILDREN

0 TO 6 MONTHS	6 TO 24 MONTHS	2 TO 5 YEARS	5 TO 10 YEARS	10 TO 14 YEARS	TOTAL
6	26	46	61	11	150

food allergy. Of the ninety-seven children with atopic eczema and perennial asthma, sixty-seven presented preliminary evidence of food sensitivity, an incidence of 71 per cent. Of the thirty-eight children who had seasonal asthma and/or hay fever, only four showed preliminary evidence of food allergy, an incidence of about 9 per cent. There were three children with urticaria and angioedema, all of whom had preliminary evidence of food sensitivity. In the miscellaneous group consisting of children with vernal conjunctivitis, insect bite allergy, seborrheic eczema, and contact dermatitis, no evidence whatever of food sensitivity was produced. In the group of four children in whom a diagnosis of asthma was not confirmed, there was one instance of suspected food sensitivity which was not confirmed.

TABLE II. PRELIMINARY EVIDENCE OF FOOD SENSITIVITY IN 150 "ALLERGIC" CHILDREN

ALLERGIC DISEASE	NUMBER OF PATIENTS	SKIN TESTS POSITIVE	HISTORY POSITIVE	SKIN TEST AND HISTORY NEGATIVE	PRELIMINARY EVIDENCE POSITIVE (PER CENT)
Perennial asthma Atopic eczema	97	56	48	30	71
Seasonal asthma Hay fever	38	3	2	34	10
Urticaria Angioedema	3	3	3	0	100
Miscellaneous*	8	0	0	8	0
Asthma not confirmed	4	0	1	3	25
Total	150				

*Poison ivy, seborrheic eczema, insect bite, vernal conjunctivitis, and contact dermatitis.

Final Evidence of Food Sensitivity in Sixty-seven Children With Perennial Asthma, Atopic Eczema, or Both.—The parents of each child were repeatedly interviewed and questioned about the ramifications of their children's food sensitivities. As a result, two groups of patients emerged: One group gave strongly positive histories which were convincing enough to be accepted at face value. In this group only a limited number of children were given test feedings. Another group gave unconvincing histories. This second group was given elimination diets and provocative feedings over and over again. Many times the provocative feedings were given at the office or clinic. This process was continued until a reasonable conclusion was reached. In almost every case, the food allergy was first suggested by the history and not by the skin tests. Wherever skin tests were positive and the history was negative, elimination diets based on the positive skin tests were tried. In only a few instances did positive food tests alone prove to have clinical significance. Final proof depended upon

production of characteristic objective allergic symptoms by test feeding on three or more ocasions at intervals of several weeks or upon an overwhelming positive history when test feeding was risky. Vague symptoms, such as nausea, cramps, or headache, were not considered reliable allergic manifestations. Test feedings were done when the children were asymptomatic for several days. When the eczema or asthma was continually severe and there was a convincing positive food history, provocative feedings were usually omitted. In most instances of definite positive food histories, mothers rejected the idea of provocative feedings. On the other hand, when the history was only suggestive, mothers would willingly accept test feedings.

There were forty children (of the sixty-seven who had preliminary evidence) with convincingly positive histories. All forty were either proved by test feedings to be food sensitive or else the food allergy was so evident or the disease so severe that provocative feeding was omitted. Some of the forty children had sensitivity to several foods, so that there was a total of 113 proved food sensitivities.

There were twenty-seven children whose histories were merely suggestive and questionable from the very beginning. Only seven of the twenty-seven were subsequently proved to be food-sensitive. The seven gave a suggestive history of thirty-seven food sensitivities, but only eight were proved. The remaining twenty-nine were unproved (Table III).

TABLE III. PROVED FOOD SENSITIVITIES IN SIXTY-SEVEN CHILDREN WITH PERENNIAL ASTHMA AND ATOPIC ECZEMA (ONE GROUP WITH CONVINCING POSITIVE HISTORIES, ONE GROUP WITH SUGGESTIVE POSITIVE HISTORIES)

	NUMBER OF CHILDREN	CHILDREN PROVED SENSITIVE	NUMBER OF FOOD SENSITIVITIES SUSPECTED	NUMBER OF PROVED FOOD SENSITIVITIES	PER CENT OF PROVED SENSITIVITIES
Convincing history	40	40	118	113	96
Suggestive history	27	7	37	8	21

SYMPTOMS PRODUCED BY FOOD ALLERGY

Although perennial asthma and eczema were the diseases from which these children suffered, the objective manifestations of their food sensitivities were not always related to their major allergic diseases. Table IV shows the symptoms produced by the foods to which sensitivity existed. In only about 30 per cent of the cases did the food seem to affect the major allergic diseases.

TABLE IV. MANIFESTATIONS OF FOOD ALLERGY IN FORTY-SEVEN CHILDREN WITH PERENNIAL ASTHMA AND ATOPIC ECZEMA

URTICARIA, ANGIO-EDEMA, AND PRURITUS	ECZEMA FLARE	WHEEZING	CONJUNCTIVITIS AND RHINITIS	VOMITING AND DIARRHEA	CANKER SORES AND FISSURES AT MOUTH	SYSTEMIC REACTIONS
23	17	17	2	10	3	3

In only one instance was a food apparently the sole etiological factor in a case of asthma. In that case, Patient S. D'L., one of twins, developed severe

asthma abruptly at the age of 3½ months. Home management, medication, and hospitalization produced no relief. Withdrawal of milk empirically resulted in dramatic relief. In the months that followed, the patient was challenged twice with milk, and each time asthma resulted. At the age of 1 year, he was able to take milk. He is now 2½ years old, and thus far there has been no recurrence of asthma. We feel that the two positive challenge feedings could have been a coincidence, but the marked improvement when the boy was off milk makes these findings hard to explain, save on the basis of an allergic mechanism.

EFFECT OF AGE

Cooke,[1] Feinberg,[2] Sherman and Kessler,[3] and Boyed[4] have stated that food allergy is more frequently encountered during infancy and early childhood and that a large percentage of the affected children lose their food sensitization spontaneously. In this study, which began in early 1955 and ended in early 1958, the sixty-seven children with atopic eczema and/or perennial asthma were shown to have 121 proved food sensitivities (Table III). Forty-five of these

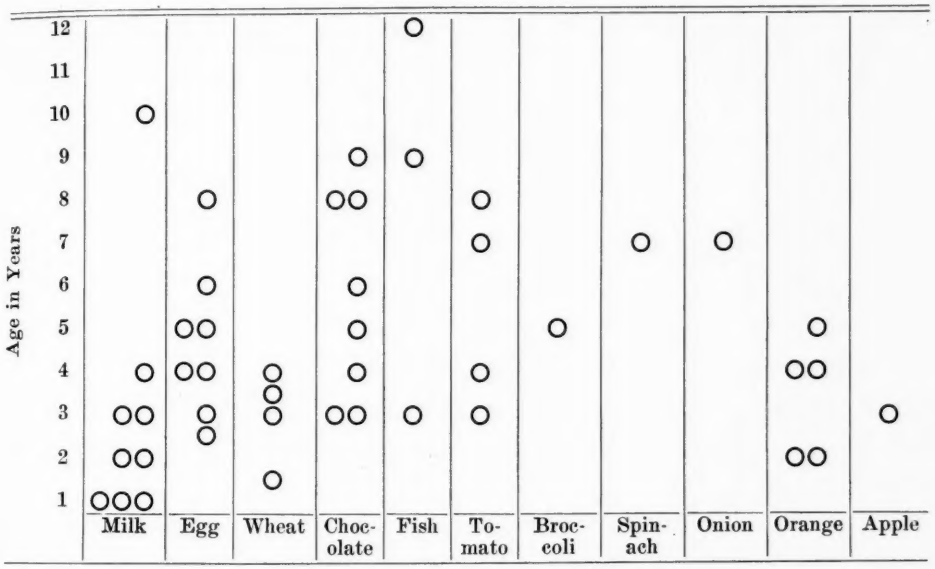

Chart 1.—Ages at which allergenic foods were tolerated. (Each circle represents one patient.)

sensitivities were lost during this period of study. Chart 1 lists the allergenic foods and the ages at which these foods appeared to be well tolerated. In a few instances in this group absolute proof of initial sensitivity was not confirmed, since tolerance developed soon after the initial visit.

CORRELATION OF SKIN TESTS WITH PROVABLE FOOD ALLERGY

Skin tests were performed by the scratch technique, using commercial 50 per cent glycerinated food extracts; in addition, we employed *natural* foods as

testing materials. The use of raw food for skin testing was suggested by Ancona and Schumacher in 1950.[5] We used raw and cooked foods for natural food testing, keeping in mind the desirability of testing with foods in the state in which they are eaten. Cottage cheese was used for a natural milk test. Egg was cooked two to three minutes. Nuts and peanuts were crushed on tinfoil before they were applied to the scratched surface of the skin. Dry foods were moistened with buffered saline. Meats and fish were tested in their raw and cooked states. The natural foods frequently gave much stronger reactions than their corresponding commercial extracts. Often the natural food tests were strongly positive when their corresponding commercial extracts gave weak positive or negative tests.

Table V shows the correlation of proved food sensitivities with results of their corresponding skin tests. It will be noted that nuts, peanuts, fish, peas,

TABLE V. CORRELATION OF PROVED FOOD SENSITIVITIES AND RESULTS OF SKIN TESTS WITH COMMERCIAL EXTRACTS AND WITH NATURAL FOODS

FOOD	NUMBER OF PATIENTS	COMMERCIAL EXTRACT		NATURAL FOOD		PER CENT OF CORRELATION (APPROXIMATE)
		POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	
Egg white	21	9	11	12	7	60
Milk	12	2	10	2	6	18
Wheat	6	3	3	3	2	60
Fish	12	7	4	10	1	90
Shellfish	3	1	1	2	0	100
Nuts	8	5	2	7	1	93
Peanut	12	8	3	11	1	92
Chocolate	15	1	14	8	6	53
Orange	7	0	6	5	1	62
Apple	1		1		1	0
Grape	1			1		100
Strawberry	1	1			1	100
Banana	2	0	1	2	0	100
Watermelon	1		1		1	0
Tomato	7	1	6	4	1	60
Pea	3	2	1	3	0	100
Onion	1				1	0
Broccoli	1				1	0
Beef	2	0	2	2	0	100
Chicken	3	1	2	0	0	33
Pork	1		1			0
Rice	1		1			0

and shellfish showed almost 100 per cent correlation between proved clinical food sensitivity and positive skin tests. Other foods showed a much lower percentage of correlation. There were 107 positive skin reactions to foods that had no clinical significance during this study. There were seventeen patients with twenty-six proved sensitivities whose skin tests were completely negative. In thirty instances, thirteen foods gave strong positive reactions, from three to five plus. Of these, twenty-one were associated with clinical sensitivity and nine were not (Table VI).

Dr. William P. Buffum[6] calls attention to the importance of a positive egg test in asthmatic infants under the age of 2 years. Twenty-five per cent

of his asthmatic babies had positive egg tests. He also states that a strong egg test usually spells a gloomy time ahead. In our series there were ten asthmatic infants seen before the age of 2. Three had 4 plus reactions to egg. All three had very severe asthma and are still sick. However, there were three others with equally severe asthma who had negative egg tests.

TABLE VI. CORRELATION OF STRONG SKIN TESTS (3+ TO 5+) TO PROVED FOOD SENSITIVITIES

FOOD	NUMBER OF PATIENTS	SKIN TESTS	PROVED	NOT PROVED
Milk	1	4+	1	
Fish	5	4+ to 5+	3	2
Pea	2	4+	2	
Spinach	1	4+	1	
Peanut	5	3+ to 5+	4	1
Wheat	3	3+ to 5+	1	2
Nut	3	4+ to 5+	2	1
Pork	1	4+		1
Lamb	1	4+		1
Beef	1	4+	1	
Chocolate	1	5+	1	
Egg	5	4+ to 5+	4	1
Raw potato	1	5+	1	
Total	30		21	9

We encountered three patients who gave negative skin reactions to natural raw fish and strong positive reactions to the same fish after it was cooked. In two instances there were negative reactions to raw spinach and strong reactions to cooked spinach. One child developed generalized urticaria from a scratch test with raw peeled potato, severe enough to require an injection of epinephrine, but that same child had a negative reaction to cooked potato. One child developed urticaria when her intact skin came in contact with wheat flour, whereas ingestion of wheat products produced no ill effects. Asthmatic symptoms appeared in one child on contact with live crabs and in several children when they were in close proximity to or came in contact with fish, raw or cooked. One child repeatedly developed an itching in the palate and vomiting upon eating egg or tuna fish after the age of 5, whereas she had tolerated these foods prior to that age

DISCUSSION

This study shows that children with atopic eczema and/or perennial asthma are likely to be sensitive to food much more frequently than those with seasonal asthma and hay fever. It also appears that food sensitivity exerts only a minor influence upon the course of atopic eczema and perennial asthma in children of this age group. In most instances the food sensitivities produced their own set of special allergic symptoms which were not related to either asthma or eczema.

The incidence of clinical food sensitivity in allergic children is subject to considerable variation. It is variable with different foods. It also varies with different age groups and with the different allergic disease entities. Glaser[7]

states that in his practice as a pediatric allergist the incidence of milk allergy is at least one in fifteen or 7.5 per cent. In 1957 Bachman and Dees[8] reported a series of 109 allergic babies under the age of 2, in whom they found a 30 per cent incidence of milk sensitivity. In our series of ninety-seven older children with nonseasonal asthma or atopic eczema, there was a 13 per cent incidence of proved milk allergy. In 1952 Ratner and Untracht[9] reported a series of 500 allergic children, in 5.6 per cent of whom they found clinical egg sensitivity. In our series consisting of ninety-seven children, all of whom were ill with nonseasonal asthma or atopic eczema, the incidence of proved egg sensitivity was about 22 per cent. Only meager information on the incidence of clinical wheat allergy in allergic children is found in the literature. In 1948, Hill[10] presented a series of 100 asthmatic children, aged 3 to 12 years in whom he found positive wheat tests in five, but not one was clinically sensitive. In 1956, in his articles on the treatment of eczema, Hill[11] states: "Wheat sensitivity is important in many eczematous babies and in not a few older children." Buffum[12] found eight children clinically sensitive to wheat in a series of forty-nine whose asthma began before the age of 2 and whose asthma was moderately severe. In our ninety-seven cases of asthma and eczema, there were six instances of wheat sensitivity, an incidence of about 5 per cent (Table V). Considering the entire series of 150 "allergic" children, the incidence of proved clinical wheat allergy was only 4 per cent.

Obviously, in making comparisons on the incidence of food allergy between different observers, one should use comparable age groups, comparable disease entities, and comparable food materials.

SUMMARY

This is a study of 150 children, all of whom were referred with a diagnosis of an "allergic" disease. Ninety-seven had atopic eczema, nonseasonal asthma, or both. Most of these patients were quite ill. Of the ninety-seven children, sixty-seven had a positive clinical food history or positive food tests. They were designated as having preliminary evidence of food allergy. These sixty-seven were observed for a period of three years. Forty-seven had proved food sensitivities. They were sensitive to a total of 121 foods. The manifestation of their food sensitivities were mostly unrelated to the asthma or eczema. Even when wheezing and eczema appeared to be due to food allergy, one could not be certain of this since these youngsters were having asthma or eczema most of the time.

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TRIAMCINOLONE IN THE TREATMENT OF ALLERGIC CONDITIONS

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THE purpose of this report is to relate our experience with the use of triamcinolone in the treatment of allergic diseases. An attempt will be made to emphasize methods of administration, clinical effectiveness, occurrence of side effects, balance studies, and any possible advantages over other steroids.

CHEMISTRY

Many synthetic steroids have recently been made available in the hope that greater glucocorticoid, mineral-corticoid, and anti-inflammatory potency may thus be obtained without the production of accompanying undesirable side effects. These side actions are modified by various changes in the chemical composition of cortisone and hydrocortisone. Thus, cortisone (Compound E) is characterized by the carbonyl oxygen at carbon 11 and the hydroxyl grouping at carbon 17. The numbers at each position indicate a carbon atom (Fig. 1). The introduction of a hydroxyl group at position 11 yields hydrocortisones or Compound F (Fig. 2). It is the principal natural glucocorticoid hormone, whereas aldosterone is the principal natural mineral-corticoid hormone. The glucocorticoid activity is an index of its anti-inflammatory property. Prednisone and prednisolone are obtained by the removal of hydrogen from positions 1 and 2, respectively (Figs. 3 and 4). This changes the properties of the product, making it much more potent. When a hydroxyl group is added at the 16 alpha position to desoxycorticosterone, the salt-retaining activity is reduced.

When the 16 alpha-hydroxyl group is added to hydrocortisone or to Δ^1 hydrocortisone (prednisolone), glucocorticoid activity is lowered, with no sodium retention.[1] The substitution of a methyl group for the hydrogen atom in the sixth alpha position produces another analogue of hydrocortisone, namely, methylprednisolone (Fig. 5). Triamcinolone is obtained by the fluorination of the product (the addition of fluorine at position 9α) and the addition of a hydroxyl group at position 16α (Fig. 6). This change reduces the sodium--retention effect produced by fluorination. Fluorination is known to increase both the mineral-corticoid and the glucocorticoid activity of the drug. Triamcinolone (Aristocort), then, is the generic term for this new synthetic corticosteroid. It is the diacetate ester of 9α Cl 19, 823.

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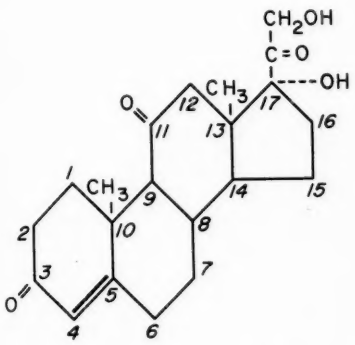
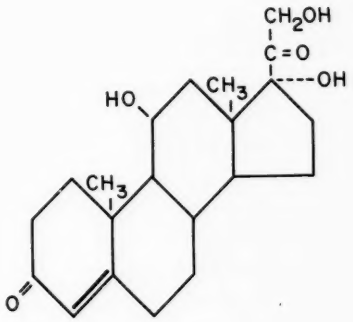
Fig. 1.



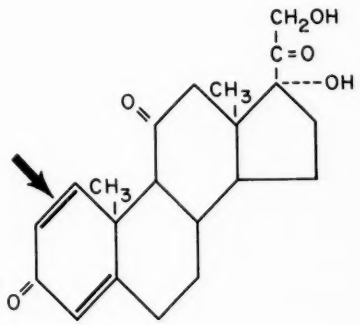
CORTISONE (COMPOUND E)

Fig. 2.



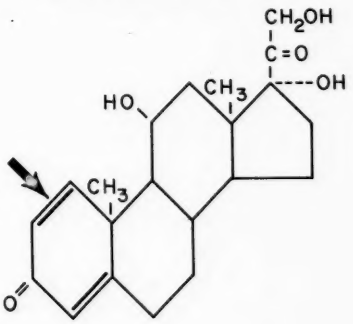
HYDROCORTISONE (COMPOUND F)

Fig. 3.



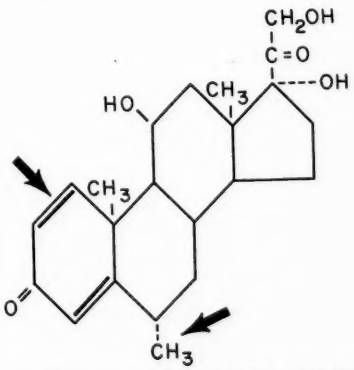
PREDNISONE

Fig. 4.



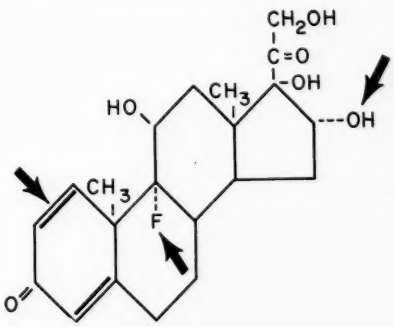
PREDNISOLONE

Fig. 5.



METHYL PREDNISOLONE (MEDROL)

Fig. 6.



TRIAMCINOLONE (ACETATE)

TABLE I.

IDENTIFICATION NO.	PATIENT (INITIALS)	CODE
Study No.		1-3
Sex	MALE (1) FEMALE (2)	4
Age	(1) 1-3 (2) 4-10 (3) 11-15 (4) 16-25 (5) 26-40 (6) 40-60 (7) 61+	5
Diagnosis	(1) PERENNIAL RHINITIS (2) HAY FEVER (3) BRONCH. ASTHMA (4) URTICARIA	6-8
	(5) ATOPIC DERMATITIS (6) SERUM DISEASE (7) DRUG ALLERGY (8) OTHER	
Disease Duration	(1) 1 YR. (2) 1-5 YRS. (3) 6-10 YRS. (4) 10 YRS. +	9
Disease Severity	(1) MILD (2) MODERATE (3) SEVERE (4) INTRACTABLE	10
Compli-cations	(1) POLYPS (2) EMPHYSEMA & FIBROSIS (3) SINUSITIS	11-12
	(4) LICHENIFICATION & INFECTION (5) OTHER	
Previous Therapy	(1) IMMUNOLOGIC (2) SYMPTOMATIC (3) STEROID	13-15
Relief on Prev. Therapy	(1) NONE (2) POOR (3) SLIGHT (4) MODERATE (5) MARKED	16
Previous Steroids:	(00) NONE CORTISONE (1) 150 (2) 300; HYDROCORTISONE (3) 100 (4) 150	17
DOSE/WK. IN MGM.	(5) METACORTIN 70 (6) 140; ACTH (7) 100 (8) 200	
Duration Previous Steroids in Wks.	(1) 2 (2) 4 (3) 8 (4) 12 (5) 16 (6) 18+	18
Previous Steroids Side Effects	(1) NONE (2) SLIGHT (3) MODERATE (4) MARKED	19
Aristocort Therapy	INITIAL DOSE IN MGM/DAY FOR 3 DAYS (1) 16 (2) 20 (3) 24	20
	DECREMENT: 2 MGM/DAY EVERY SECOND DAY	
	DAILY MAINTENANCE IN MGM (1) 2 (2) 4 (3) 6 (4) 8 (5) 10	21
	DURATION OF THERAPY IN WEEKS (1) 1-2 (2) 3-8 (3) 8-12 (4) 12-18 (5) 18+	22
	RELIEF (1) NONE (2) SLIGHT (3) MODERATE (4) MARKED	23
SIDE EFFECTS	24 C N S INSOMNIA 25 TREMORS 26 HEADACHE 27 EUPHORIA 28 CONVULSIONS	24-28
	29 DEPRESSION 30 VERTIGO 31 SWEATING 32 FLASHES 33 IRRITABILITY	29-33
	34 CARDIO VASC. FAILURE 35 HYPERTENSION	34-35
	36 G.I. ULCER SYMP. 37 CRAMPS 38 FLATULENCE 39 INCR. APPETITE	36-39
	40 G.U. FREQUENCY 41 NOCTURIA 42 LIBIDO CHANGE	40-42
	43 ENDOCRINE WT. CHANGE 44 EDEMA FAT 45 DEP. CHANGE 46 HIRSUTISM	43-46
REMARKS		

			CODE
SIDE EFFECTS (CONT.)	ENDOCRINE	47 MOON FACIES 48 PURPURA 49 STRIAE 50 ACNE	47-50
		51 DRYNESS 52 FATIGUE	51-52
	INFECTION	53 VIRAL 54 BACTERIAL 55 TUBERCULOSIS	53-55
	G. I.	56 HEMORRHAGE 57 ULCER	56-57
COMPLI-CATIONS	ENDOCRINE	58 DIABETES 59 ADRENAL CRISIS	58-59
		60 CUSHINGS SYNDROME 61 GROWTH	60-61
		62 GROWTH ARREST 63 OSTEOPOROSIS THYROID ENLARGE.	62-63

Lab. Results	(1)	(2)	(3)	(4)	CODE
HEMOGLOBIN	NO CHANGE	INCREASE	DECREASE		64
HEMATOCRIT	NO CHANGE	INCREASE	DECREASE		65
W B C	NO CHANGE	INCREASE	DECREASE		66
EOSINOPENIA	NONE	MILD	MARKED		67
CHEST X-RAY	NORMAL	CHANGE	ABNORMAL		68
TUBERCULIN TEST	POSITIVE	CHANGE	NEGATIVE		69
GLYCOSURIA	NO CHANGE	INCREASE	DECREASE	NONE	70
GLUCOSE INTOLERANCE	NONE	MILD	MARKED		71
N P N	NO CHANGE	INCREASE	DECREASE		72
CHOLESTEROL ELEVATION	NONE	MILD	MARKED		73
HYPONATRIURIA	NONE	MILD	MARKED		74
HYPERCALCIURIA	NONE	MILD	MARKED		75
HYPOKALEMIA	NONE	MILD	MARKED		76
TOTAL PROTEIN	NO CHANGE	INCREASE	DECREASE		77
GAMMA GLOBULIN	NO CHANGE	INCREASE	DECREASE		78
VITAL CAPACITY	NO CHANGE	INCREASE	DECREASE		
TIMED VITAL CAPACITY	NO CHANGE	INCREASE	DECREASE		79
MAXIMUM BREATHING CAPACITY	NO CHANGE	INCREASE	DECREASE		80

LITERATURE

There is abundant literature dealing with the effectiveness of steroids and corticotropin in the symptomatic treatment of allergic conditions.[2-7] Perrine and associates[8] have shown that triamcinolone is 10 to 40 times more active than hydrocortisone and 3 to 12.3 times more active than prednisolone in producing glycogen deposition in fasted adrenalectomized rats. Sherwood and Cooke[9] recite their experience with this drug in a preliminary report. Feinberg and colleagues[10] state that triamcinolone is potent and effective in the treatment of seventy allergic patients in about one-half the dose of prednisone. Their findings indicate that it has a diuretic and sodium-depleting effect. Rein and his associates[11] find it to have "potent anti-inflammatory and antipruritic properties" without marked side effects in twenty-six patients with dermatologic conditions. Triamcinolone has also been used satisfactorily in the treatment of rheumatoid arthritis.[12] In this study it is found that it is a powerful anti-inflammatory agent with a potency equal to prednisone and prednisolone and that it produces fewer side effects than other steroids. Potency and side effects produced by triamcinolone, especially leg cramps and sweats, are discussed in several other papers.[13-15] Dubois,[16, 17] in recent accounts, indicates triamcinolone to be effective in the treatment of twenty-nine patients with systemic lupus erythematosus. It brought about a weight loss of 7.8 per cent in eighteen patients. According to this report, hirsutism, striae, and Cushingoid appearance are more frequent and more marked than when other steroids are used. Muscle weakness is predominant. However, some patients are better controlled with triamcinolone than with other steroids. Balance studies have been reported.[18-20] When triamcinolone is given in daily doses of 12 mg., both sodium loss and water loss occur. None of the patients show hypokalemia, hyperkalemia, hypochloremia, or a change in calcium excretion.[12] Positive nitrogen balance is well maintained.[13]

METHOD OF STUDY

The allergic patients who form the basis for these observations were obtained from various sources: private practice, the allergy wards of the U. S. Veteran's Hospital, the allergy clinics of the University of Pittsburgh School of Medicine, Montefiore Hospital, and the Veterans Administration. The clinical effect of triamcinolone was observed by five different physicians, and the results were tabulated by them. Thus, the final picture is a composite of all these reports. A schedule of dosage and frequency of administration was given to each patient. The patients were seen at frequent intervals. Work sheets were kept on each case, summarizing the clinical data (history and physical examination) and laboratory findings. In so far as it was possible, laboratory studies included routine urine examinations, complete blood counts, chest roentgenograms, blood chemistry, electrolyte studies, and pulmonary function tests. Each test was repeated after the patient had received triamcinolone for a given period of time. These findings were then tabulated. The summary sheet included pertinent data as to clinical and laboratory findings (Table I), together with such additional data as previous administration of

steroids and the side effects noted, the side effects and complications which followed the administration of triamcinolone, etc. After proper coding, this information was processed through an IBM machine and the results were tabulated.

A total of 250 allergic patients were treated with triamcinolone. This includes 119 males and 131 females. As for age distribution, there were twelve patients aged 1 to 3, twenty-seven patients aged 4 to 10, twenty-three patients aged 11 to 15, thirty-six patients aged 16 to 25, seventy-one patients aged 26 to 40, seventy-one patients aged 40 to 60, and ten patients over 61 years of age. In many instances patients presented more than one type of allergy, that is, asthma and hay fever or asthma and atopic dermatitis. There were fifty-three instances of perennial nasal allergy, fifty-four of seasonal hay fever, eighty-nine of bronchial asthma, twenty-five of urticaria, eighty-three of atopic dermatitis, four of serum disease, twelve of drug allergy, and eight of contact dermatitis. The duration of the disease varied. It was present in thirty-five instances for one year, in sixty-seven for one to five years, in sixty-eight for six to ten years, and in eighty for a period of more than ten years. Complications such as nasal polyps were found associated with nasal allergy in sixteen patients and sinusitis was found in eleven patients; fourteen asthmatic patients showed evidence of pulmonary emphysema, pulmonary fibrosis, and chronic bronchitis; fifty-six patients with atopic dermatitis developed marked secondary skin changes; thirty-five showed the presence of a combination of these complications; and in one hundred eighteen patients there were no complications. A total of seventy-two patients out of the 250 in this series received previous steroid and/or corticotropin therapy for a period of four to twelve weeks with excellent therapeutic results. Of these, four received cortisone, four received hydrocortisone, forty-one received metacorten, twelve received ACTH, and eleven received a combination of more than one of these products.

TABLE II. DURATION OF THERAPY WITH TRIAMCINOLONE

DURATION (WEEKS)	MALES	FEMALES	TOTAL
1- 2	11	11	22
3- 8	44	39	83
8-12	27	33	60
12-18	18	29	47
18+	19	19	38
Totals	119	131	250

Triamcinolone was given daily in divided doses. The initial dose was as follows: 206 patients received 16 mg. per day, nine patients received 20 mg. per day, and thirty-five patients received 24 mg. per day. The dose was reduced by 2 to 4 mg. per day every one to two days. The maintenance dose was 2 mg. per day in 155 patients, 4 mg. per day in seventy-one patients, and 6 to 10 mg. per day in twenty-four patients. Only one patient required 12 mg. per day as a maintenance dose. The drug was discontinued gradually. No sodium-restricted diet or potassium was prescribed. Triamcinolone was

administered over varied periods of time (Table II), ranging from two to eighteen weeks. When patients received a small maintenance dose, this was given in the mornings, for it has been shown that it is then that adrenal cortical activity is lowest. Usually other antiallergic management was continued during and after steroid medication, thus making it possible to employ smaller doses of triamcinolone. Antacid and antiulcer medication was given to only a few patients in the series. Relief from steroids in allergic conditions is a well-established experience, which is further corroborated by our results in this series. The clinical improvement following the administration of triamcinolone is dramatic and compares favorably with that obtained by us[21] and others[22] with Meticorten. It is to be pointed out that, even in the presence of the usual conditions complicating long-standing allergy, clinical results were better when triamcinolone was added to other therapy.

TABLE III. LABORATORY STUDIES AS TO EFFECT OF TRIAMCINOLONE

NATURE OF TEST	NO. OF PATIENTS TESTED	NO CHANGE	INCREASE	DECREASE
Hemoglobin	209	208	0	1
Hematocrit	208	205	2	1
W.B.C.	201	138	8	55
Eosinopenia	203	47	61	95
Chest x-ray	189	189	0	0
Tuberculin	155	57	0	98
Glycosuria	190	186	4	0
Glucose tolerance	183	178	0	5
N.P.N.	176	176	0	0
Cholesterol	121	119	2	0
Hyponatriuria	12	10	2	0
Hypercalciuria	9	9	0	0
Hypokalemia	8	8	0	0
Total protein	34	33	0	1
Gamma globulin	8	8	0	0
Timed vital capacity	47	15	32	0
Maximum breathing capacity	38	11	27	0

Laboratory studies were carried out as indicated in Table I in most, but not all, of these 250 patients. For the most part, these showed no marked changes as a result of triamcinolone therapy in maintenance doses of 2 to 4 mg. per day (Table III). The hemoglobin, hematocrit, and red cell count were unaffected. Eosinopenia occurred in ninety-five of the 203 patients in whom the count was done. The chest x-ray was unaffected. Four patients developed glycosuria, and five patients showed a suggestive diabetic sugar-tolerance curve. The tuberculin test was diminished in ninety-eight out of 155 patients. We found hypercholesterolemia in two patients and hyponatriuria in two. No change in nonprotein nitrogen was seen. There was no evidence of hypercalciuria, hypokalemia, or significant changes in total protein or gamma globulin. The timed vital capacity and the maximum breathing capacity were increased in asthmatic patients. An attempt was made to determine whether daily doses greater than the maintenance dose given the patients in this study, namely, 18 to 24 mg., would cause any metabolic disturbance. Six patients were studied by two different observers. All had

bronchial asthma of long standing. Base line studies were performed while the patients were on 10 mg. of triamcinolone. The patients were placed on a 500 mg. sodium diet. After they had been on this for four days, 12 Gm. of sodium chloride was administered for three days and twenty-four-hour urine sodium and calcium excretions were performed. The calcium studies were done in six days; 18 to 24 mg. of the drug was given daily, and this was kept up throughout the metabolic study (Table IV). An analysis of Table IV shows only slight changes in urine sodium and calcium findings. Thus, patients 3 and 5 showed mild sodium and water retention. Patient 2 also showed a slight increase in cholesterol and a decrease in basal metabolic rate, but the PBI determination did not reflect this.

TABLE IV. METABOLIC (BALANCE) STUDIES

PATIENT	AVERAGE EXCRETION IN URINE SODIUM	Q CALCIUM (MEG./LITER/DAY)	URINE IN 24 HOURS (C.C.)
1. Before drug	78	4.9	1,412
On drug	169	3.2	1,400
2. Before drug	215	5.1	2,700
On drug	182	4.9	2,300
3. Before drug	192	4.3	2,600
On drug	178	4.2	2,410
4. Before drug	94	3.9	2,840
On drug	103	3.2	2,600
5. Before drug	186	4.5	3,400
On drug	153	3.4	3,200
6. Before drug	204	4.6	2,400
On drug	193	4.1	2,350

Table V summarizes the nature of the side effects and the complications following administration of triamcinolone. These are dependent largely on the size of the daily dose and the duration of treatment. Nervous manifestations, including insomnia, tremors, headache, euphoria, depression, vertigo, increased irritability, and weakness were evident in fifty-nine patients. Of

TABLE V. SIDE EFFECTS FROM TRIAMCINOLONE THERAPY

SIDE EFFECTS	NO. OF PATIENTS
Nervous manifestations:	
Insomnia, tremors, headache, euphoria, depression, vertigo, irritability, and weakness	59
Sweating and flashes	58
Cardiac failure	0
Hypertension	3
Gastrointestinal (ulcer)	15
Gastrointestinal hemorrhage	0
Ulcer proved by x-ray	3
Cramps in extremities	34
Urinary frequency and nocturia	73
Loss of weight (2 to 4 lbs.)	89
Continued loss of weight	2
Buffalo hump	5
Moon facies	22
Striae	4
Acne	9
Erythema of face	8
Hirsutism	9

this number, twenty-one had more than one manifestation. Sweating and disturbing flashes were seen in fifty-eight patients. There were no instances of cardiac failure. Hypertension of a moderate degree was evident in only three instances. Fifteen patients developed ulcer symptoms which did not exist previously. These were confirmed as peptic ulcer by gastrointestinal x-ray series in three cases. Two of these fifteen patients had received Meticorten previous without gastric upset. On the other hand, four patients who had severe gastric irritability previously following Meticorten could take triamcinolone without any disturbance. Unexplainable cramps in the extremities occurred in thirty-four patients. About one-third of the entire group of 250 patients reported increased urinary frequency and some nocturia. About an equal number lost an average of 3 pounds in weight during the first few days on triamcinolone. This is obviously due to loss of water. Two patients continued to lose weight (10 to 18 pounds), and in these patients the drug was discontinued. Other side effects noted were as follows: buffalo hump in five instances, moon facies in twenty-two, hirsutism in nine, striae in four, acne in nine, and erythema of the face in eight. There were no complicating infections and no instances of adrenal crises, growth arrest, or thyroid dyscrasia. Only one patient developed osteoporosis, and this patient received a maintenance dose of 20 mg. per day. Most of the more serious side effects occurred when the dose of the drug exceeded 10 mg. per day. In some cases, it was difficult to determine whether symptoms which occurred during the administration of the drug were coincidental with or related to the drug. One patient, a 21-year-old asthmatic girl, became depressed and irritable when she received ephedrine and triamcinolone. It was not clear which of the two preparations upset her. Subsequently, it developed that the emotional difficulty was due to the steroid. This patient had a similar effect from Meticorten.

Twelve patients received Equanil, 200 mg. every six hours, in addition to triamcinolone. Similar combinations have been used by others.[23] In three of these patients the reduction of emotional tension was helpful. We did not find it possible to reduce the dose of triamcinolone because of the addition of the tranquilizing agents.

Triamcinolone was employed in three of our patients who had severe bronchial asthma and mild diabetes mellitus. The steroid, given in maintenance doses of 2 mg. per day, did not affect the diabetic therapeutic program. In another patient who had moderately severe diabetes it was necessary to increase the dose of insulin.

We had occasion to employ the drug in three cases of severe constitutional reactions following the administration of pollen extract. It was given together with the usual procedures used in the treatment of such a reaction. Unless given intravenously, no corticosteroid has an immediate effect on a constitutional reaction, whether of the anaphylactic or of the atopic type.

Massive or larger doses have been used by us in five cases of status asthmaticus, starting with 70 mg. per day for the first two days and gradually reducing the dose. There is no question as to its effectiveness in releasing

bronchospasm and reducing bronchial obstruction. The latter effect may well be due to its action on the viscid sputum. In two instances, however, the therapeutic effect was not very rapid. Other measures had to be adopted to give the patient relief. This only points out the fact that mechanical obstruction and infection in the bronchi cannot always be relieved by the administration of triamcinolone or of any other steroid. The effect of triamcinolone may well be at the cellular level. In all probability, it is due to its anti-inflammatory effect and to interference with the production of histamine and of other products which result from the antigen-antibody union but not to any effect on antibody formation or on the antigen-antibody union. Excessively large doses have been reported to interfere with antibody production in the experimental animal[24, 25] but not in man.[26]

The eosinopenic potency of triamcinolone, while unquestionably higher than that of hydrocortisone, is approximately the same as that shown by us in connection with Meticorten.[21]

The average maintenance dose in rheumatic conditions, according to Hartung,[12] was 10 mg. per day in divided doses, an amount considerably higher than that needed, in our experience, in allergic conditions. With such dosage, most of the patients in Hartung's series showed some side effects although these were, for the most part, mild. Blodgett and associates[27] draw attention to the fact that low dosage is less likely to lead to growth arrest. This is also true of other complications and side effects.

An analysis of our experience with triamcinolone as well as with other corticosteroids leads to the following impressions:

Triamcinolone is about 1.5 times as potent as prednisone (Meticorten) as an antiallergic agent. This increased potency may be an advantage if the cost of the drug, milligram for milligram, is the same as that of other corticosteroids. It would further appear that side effects resulting from its administration are about the same as those to be expected from other synthetic steroids. However, triamcinolone gives rise to fewer instances of hypertension and severe gastrointestinal symptoms. Common side effects found in our series are weakness, sweats, and muscle cramps. In the dosage used by us there certainly is an absence of sodium retention, potassium depletion, and edema. It commonly causes diuresis. Because of the absence of these early warnings, it becomes even more important to be on the lookout for possible dangerous complications. In other words, freedom from side effects does not render the drug safe.[28, 29]

The number of patients receiving triamcinolone in this study is relatively large. This, however, should not be taken as an indication of the frequency with which we approve of its administration in allergic patients. As with all corticosteroids and with corticotropin, there are real dangers accompanying the long-term use of triamcinolone.[30] These are so well known that it is not necessary to discuss them here. The patient needs to be checked periodically while taking the drug. Only the smallest amount of drug needed to keep him comfortable should be employed. Patients are urged to be conservative in the use of the drug, and the possible dangers of long-term use

should be explained to them. Most allergic patients do well with usual anti-allergic therapy and do not need steroids. In my opinion, triamcinolone, as well as other corticosteroids, is indicated in the treatment of allergy only when it is used under the following conditions: (1) purely as a temporary measure in acute transient disorders, such as ivy poison dermatitis; (2) when it is impossible to control disabling manifestations, such as intractable bronchial asthma, in spite of a complete medical and allergic study and management; (3) as a crutch during the period in which medical and allergic studies are being conducted if other forms of symptomatic therapy are inadequate; (4) in the absence of any of the well-known contraindications to steroids; and (5) as a lifesaving procedure in status asthmaticus. This therapy should be undertaken on a long-term basis only after a careful appraisal of potential dangers as well as benefits. It is not to be used as a substitute for careful medical and allergy management. Particular caution is to be exercised in the long-term treatment of allergic children.

SUMMARY AND CONCLUSIONS

Triamcinolone has been used in the treatment of 250 actively allergic patients. The drug is potent and gives marked clinical relief. It is somewhat more potent than Meticorten. The frequency and severity of side reactions is about the same as observed with other synthetic steroids, except perhaps for fewer instances of hypertension and gastrointestinal manifestations. Weakness, sweats, and muscle cramps are somewhat more common. The drug produces sodium excretion and diuresis. A note of caution is included with reference to the use of triamcinolone and other steroids in the long-term treatment of allergy.

The author wishes to express his thanks to Philip Blank, M.D., who supplied some of the patients and observations used in this study, and to his residents—John C. Winter, M.D., Francis X. Dufault, M.D., M. James Folston, M.D., and Joseph Guter, M.D.—who aided in obtaining data on clinical patients.

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BIGELOW APT.

TREATMENT OF STATUS ASTHMATICUS COMPLICATED BY VASOMOTOR COLLAPSE

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MEASURES employed in the treatment of the acute severe attack of asthma or of the more prolonged status asthmaticus are quite clearly delineated in textbooks and journals. Except for the occasional patient who has severe and intractable obstruction to air exchange, the person with asthma rarely is faced with the threat of immediate death.

The sudden development of vasomotor collapse and profound shock in a patient suffering an acute severe bout of asthma prompted us to utilize a form of therapy not heretofore reported in the management of asthma. In this instance, measures to combat shock took precedence over other elements of treatment. Relief of anoxia and carbon-dioxide retention and eradication of infection were integrated to provide an effective therapeutic program. We wish to emphasize the importance of prompt and meticulous treatment of shock as the keystone of therapy in any situation in which it is a complication.

CASE REPORT

On the morning of May 31, 1958, a 20-year-old female student had an episode of severe bronchial asthma and was admitted to the Palo Alto Hospital.

Present Illness.—Mild nonseasonal asthma had been present from childhood to the age of 17 years. During the past three years the patient had been almost completely free of asthma. During the week prior to admission, she experienced a recurrence of asthma, for which she required epinephrine 1: 100 by nebulization. On the day before her admission to the hospital, the asthma became more severe in spite of the use of ephedrine and amytal. She was relieved briefly after administration of 2 minims of epinephrine 1:1,000 subcutaneously, 4 mg. of Medrol orally, and 0.2 Gm. of Seconal. During the night she was given aminophylline, 500 mg. intravenously, and 1,000,000 units of penicillin intramuscularly. Because of increasingly severe respiratory distress, she was admitted to the hospital.

Past History.—At 6 weeks of age the patient developed diffuse infantile eczema, and at the age of 2 years she had atopic eczema.

Family History.—The patient's paternal grandfather and her father had asthma. Her sister has hay fever.

Physical Examination.—On admission, the patient was an acutely ill, cyanotic young woman in great respiratory distress. Her temperature was 97° F., respirations were 60 per minute, pulse rate was 100 per minute, and blood pressure was 130/60 mm. Hg. She was semistuporous and responded only to painful stimuli. The thorax was hyperinflated, and breath sounds were markedly diminished bilaterally. Faint high-pitched expiratory and inspiratory wheezes were audible. The remainder of the physical examination was not remarkable.

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Admission Laboratory Data.—The hematocrit was 55 per cent. The hemoglobin was 15.6 grams per 100 ml.; the white count was 41,000 per cubic millimeter with 95 per cent neutrophilia. A voided urine specimen contained 4+ protein and 15 to 20 white cells per high-power field. The venous serum CO_2 content was 27.2 mEq. per liter; serum sodium was 139 mEq. per liter; and serum potassium was 4.5 mEq. per liter. Chest x-ray showed no evidence of underlying pulmonary disease. An electrocardiogram demonstrated a sinus tachycardia with rate of 150 beats per minute and tall peaked P waves.

Hospital Course.—Oxygen by mask at the rate of 7 liters per minute was started immediately. Aminophylline, 500 mg. was given intravenously over the space of ten minutes. This was followed immediately by hydrocortisone hemisuccinate, 200 mg. intravenously. An infusion of 10 per cent invert sugar containing 55 mEq. of sodium and 23 mEq. of potassium per liter was started. To this was added an additional dose of hydrocortisone hemisuccinate, 200 mg. Although pulmonary ventilation improved and cyanosis disappeared, air exchange remained poor and stupor persisted. Periodically it was necessary to aspirate secretions which accumulated in the trachea. For six hours there was little change in the patient's condition, but suddenly she manifested signs of profound shock. Her blood pressure was unobtainable, her skin was cold and mottled, and she appeared in extremis.

With the onset of shock, the program of therapy was altered to include a vasopressor agent. Four hundred milligrams of metaraminol bitartrate (Aramine) was added to 500 ml. of the electrolyte solution which already contained 100 mg. of hydrocortisone hemisuccinate. It was necessary to administer this solution intravenously at a rate of 40 drops a minute to maintain the systolic blood pressure at 100 mm. Hg. Because of the possibility that bacteremia might be responsible for the development of shock, 500 mg. oxytetracycline was added to the infusion.

During the next six hours the patient recovered consciousness and could understand simple commands. Two hours later the concentration of metaraminol in the infusion was reduced to 400 mg. per 1,000 ml. Respiration improved, but although the respiratory rate decreased to 40 per minute, evidence of bronchial obstruction persisted. Endotracheal aspiration with a catheter was difficult. Anticipating only a brief period during which aspiration would be required, an endotracheal airway was employed instead of a tracheotomy. Isuprel 1:100, nebulized into the endotracheal tube brought about further improvement.

After fourteen hours of continuous administration of vasopressor, the concentration of the drug was reduced for the ensuing four hours. At this time the patient was alert and cooperative. She had improved sufficiently to permit withdrawal of the vasopressor and oxygen and removal of the endotracheal airway. She was able to take fluids by mouth, 250 mg. tetracycline four times daily and 6 mg. Medrol four times daily. The white count was 15,300 per cubic millimeter; urinalysis again revealed a 3+ albumin. Serum electrolyte concentrations were normal. An L. E. preparation was negative. The dose of steroid was gradually decreased, and other medication was discontinued. The patient was discharged on the seventh hospital day, asymptomatic and with no abnormal physical or laboratory findings.

DISCUSSION

The cause of the onset of the severe attack of asthma and of the collapse which accompanied it is unknown. Initially, coma was thought to be the result of respiratory insufficiency and sedation. Fever and leukocytosis were interpreted as signs of infection, and, with the advent of shock, bacteremia seemed likely. The combination of asthma, fever, leukocytosis, proteinuria, and coma was compatible with a fulminant exacerbation of collagen disease. Anoxia and respiratory acidosis were so mild that it seemed unlikely that coma would result. The serious complications of asthma in this patient may have been the result of sensitivity to penicillin.

Whatever the origin of this episode, the treatment employed was appropriate for all causes mentioned above. It was our purpose to combat the three components of the patient's illness which threatened life—shock, respiratory insufficiency, and infection.

Treatment Employed for Shock.—The treatment of shock included (a) hydrocortisone in large doses, (b) appropriate amounts of fluid containing sodium and potassium salts, and (c) a vasopressor agent, metaraminol.

(a) In patients such as this there are three reasons for employing adrenal corticosteroid: (1) Hydrocortisone is useful to combat shock per se. Although the occurrence of acute adrenal insufficiency is rarely the cause of collapse, the efficacy of supplementing endogenous corticosteroid secretions when vasomotor collapse occurs in times of severe stress has been well established. (2) The steroid potentiates the effect of pressor amine used simultaneously. It has been clearly demonstrated that cortisol restores "responsiveness" to vasopressor amine (norepinephrine) when response to the vasopressor agent alone is inadequate.[1-3] (3) Hydrocortisone is useful as an antiasthmatic drug.

We employed hydrocortisone hemisuccinate (Solu-Cortef) in relatively large doses. Two hundred milligrams of hydrocortisone (275 mg. of the hemisuccinate) was given intravenously, followed by an intravenous drip at a rate of 25 mg. per hour. Subsequently, the dose was reduced to 12 to 15 mg. per hour in the continuous infusion.

(b) The use of sodium-containing solutions in treatment of shock is conventional. The efficacy of potassium in the treatment of the vasomotor instability and the reduced cardiac output of hypokalemia is well established, though not widely known. Although the patient had no overt signs of hypokalemia, our experience confirms that of Hartman,[4] namely, that patients ill with severe asthma of any duration suffer from negative potassium balance as a result of inadequate intake and the unusual losses incident to stress of illness and the use of epinephrine. Furthermore, the use of glucose infusions often produces a redistribution of potassium so that there follows a low concentration of potassium in extracellular fluid. To ensure adequate total body stores and extracellular fluid concentration of potassium, 25 to 50 mEq. was included in each eight-hour infusion.

(c) The sudden development of profound vasomotor collapse may require prompt and vigorous use of vasopressor substances in conjunction with hydrocortisone.

Metaraminol bitartrate (Aramine) was selected since it is a potent vasopressor relatively free of undesirable effects on the heart and kidney and has a more prolonged duration of action than norepinephrine.[5] A further advantage is that it does not produce necrosis when injected subcutaneously or intramuscularly.[6] Metaraminol has been employed successfully in the treatment of shock accompanying myocardial infarction, trauma, surgery, and a variety of other conditions.[7-11]

For intravenous administration, concentrations of 50 to 800 mg. of metaraminol bitartrate per liter of electrolyte- or glucose-containing solution are used. The successful treatment of shock requires administration of vasopressor drug at a rate which will maintain blood pressure at desirable levels. The concentration of vasopressor drug is determined by the patient's fluid requirement. Success depends precisely on the use of the required quantity of vasopressor per minute contained in the desirable minute volume of infusion fluid. Continuous and close attention to the patient is necessary; intermittent or casual attention to the patient's needs is less likely to produce a successful result.

In this instance, in view of hyperpnea and fever, we estimated fluid requirement at 1,000 to 1,500 ml. per six hours. Metaraminol bitartrate (Aramine) was initially effective in sustaining blood pressure at a dose of 2.0 mg. per minute or a concentration of 800 mg. per liter! Within three hours the dose was reduced to 1.0 mg. per minute, so that at a flow of 2 to 2.5 ml. per minute the concentration of metaraminol required was only 400 mg. per liter. By seven hours after the beginning of therapy, the concentration of metaraminol was reduced to 150 mg. per liter, providing a dose of 0.4 mg. per minute. All infusions contained hydrocortisone, 100 mg. per liter.

Treatment Employed to Sustain Ventilation.—Only during the first four hours of treatment in the hospital was the patient cyanotic. Conventional therapy with aminophylline intravenously and epinephrine subcutaneously plus hydrocortisone administered intravenously alleviated bronchiolar obstruction to the extent that, with oxygen by mask at a flow rate of 6 liters per minute, anoxia was relieved. Clinically, there was no sign of serious respiratory acidosis. Fluids and hydrocortisone and, later in the course of therapy, metaraminol apparently contributed to improvement in pulmonary exchange. With the development of vascular collapse and deepening coma, cough was inadequate to clear the trachea and bronchi, and partial obstruction of the upper respiratory tract became a serious matter. Initially, intermittent aspiration with a catheter passed to or just beyond the larynx served to remove secretions satisfactorily. Subsequently, the need for tracheotomy or its equivalent became clear. However, because of improvement in other aspects of the patient's illness and because of the conviction that only a few hours of tracheobronchial suction would be required, an endotracheal airway was inserted instead. This facilitated the use of aspiration to clear the trachea of secretion and provided a means of administering Isuprel, 1 : 100, by nebulization.

Treatment of Infection.—Fever and leukocytosis were prominent signs, the cause of which was never determined. Blood culture was sterile, and sputum cultures contained only ordinary bacterial flora. There was no evidence of pneumonitis. Since infection was a possible cause of the initial exacerbation of asthma and of the collapse which followed, antibiotic therapy was mandatory. Oxytetracycline, 500 mg., was added to each of two liters of fluid administered during the period of shock. Subsequently, tetracycline, 1 Gm. per day, was administered orally.

SUMMARY

The case of a patient who developed severe shock as a complication of status asthmaticus is described. In addition, clinical findings of fever and leukocytosis suggested that underlying infection was a contributory factor. Treatment was designed to combat the three principal threats to life, namely, shock, respiratory insufficiency, and infection. For shock, a vasopressor amine was used in conjunction with hydrocortisone hemisuccinate and appropriate fluid replacement. For treatment of respiratory insufficiency and asthma, oxygen, hydrocortisone hemisuccinate, fluids, and removal of tracheal secretions through an endotracheal airway were employed after conventional measures had proved inadequate. For infection, oxytetracycline was added to the solutions already in use. The successful result of this integrated therapeutic program justifies its further use in similar situations.

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THE CLINICAL EVALUATION AND THE PREPARATION AND STANDARDIZATION OF SUSPENSIONS OF A NEW WATER-INSOLUBLE WHOLE RAGWEED POLLEN COMPLEX

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THE first extracts made from pollen were prepared by extracting the whole pollen grains with either water,[1] N/200 sodium hydroxide and 0.9 per cent sodium chloride solution,[2] 66 per cent glycerol solution,[3] phenol and pollen suspended in buffered salt solution,[4] or 5 per cent dextrose[5] (Table I). Milford[6] and Moore and associates,[7] in carefully controlled experiments, showed that there is an allergenically active constituent in pollen oil. Most common pollens contain considerable quantities of water-immiscible oil. Because of the presence of this water-immiscible oil, extraction of the whole pollen with aqueous extracting fluids results in turbid solutions. Therefore, in 1922 Coca[8] suggested removing the oily substances with ether, and in 1931 Stull and his co-workers[9] removed fatty material from the pollen to facilitate aqueous extraction. Since that time, all the various modifications of the preparation of pollen extracts have started with defatted pollen[10-19] and have been based upon the following statement by Stull, Chobot, and Cooke[9]: "The skin-active material from defatted pollen was practically insoluble in ethyl ether, petroleum ether, chloroform, benzene and absolute alcohol." Although it is obvious that an aqueous extract of pollen which before extraction has been rendered fat-free will not contain the fatty fraction, it has been erroneously assumed from the work of Stull and associates that there is no skin reactivity in the fatty fraction. Their pollen had been completely defatted before aqueous extraction, so it would have been most unlikely that any oil or activity remained in the pollen for subsequent extraction from an aqueous extract into a fat solvent.

Rockwell[18] has suggested that an active fraction in ragweed pollen is a proteose, carbohydrate, flavonol complex. Extraction of pollen with an organic solvent, such as ether or acetone, which dehydrates as it defats would not only tend to disrupt this complex by extracting the flavonol but could also change the nature of the original proteose due to dehydration at room temperature. Many laboratories actually use a Soxhlet extractor to remove all oil from the pollen, and this procedure is carried out at approximately 35° C. for hours. When proteins are isolated by precipitation with alcohol or

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acetone, this is carried out in most instances in a cold room at 5° C. or lower to prevent changes in the protein moiety. Table I shows variations in the preparation of pollen extracts.

TABLE I. VARIOUS PROCEDURES IN THE PREPARATION OF POLLEN EXTRACTS

STATE OF POLLEN BEFORE EXTRACTION	EXTRACTING FLUID AND ADDITIONAL PROCEDURES	AUTHOR	DATE
Not defatted	Water	Noon	1911
Not defatted	N/200 NaOH and 0.9% NaCl solution	Cooke	1915
Not defatted	33% saturated NaCl solution and 66% glycerol	Clock	1917
Defatted	Coca's solution	Coca	1922
Not defatted	Olive oil, glycerin, and petroleum	Milford	1929
Not defatted	5% Dextrose and 0.1% cresol	Moore et al.	1932
Not defatted	Phenol, then pollen suspended in buffered salt solution	Murphy	1929
Defatted	Coca's solution	Stull et al.	1930
Defatted	Aqueous* solution, then formalin	Carter	1936
Defatted	Aqueous, then ultrafiltered	Spain et al.	1935
Defatted	Aqueous, then alum	Zoss, et al.	1937
Defatted	Aqueous, then Lanolin and olive oil	Naterman	1938
Defatted	Aqueous, then formalin	Stull et al.	1940
Defatted	Aqueous, then heat		
Defatted	Aqueous, then acetylation		
Defatted	Aqueous, then ultraviolet radiation		
Defatted	Aqueous, then alum		
Defatted	Aqueous, then gelatin	Spain et al.	1941
Defatted	Aqueous, then frozen, dried, in sesame oil	Taub et al.	1941
Defatted	Aqueous, then tannic acid	Naterman	1941
Defatted	N/100 NaOH in saline solution, HCl precipitated	Rockwell	1953
Defatted	Aqueous, then Z_nCl_2, tannic acid, formalin, and Al $(OH)_3$	Naterman	1957

*Referring to bicarbonate or sodium chloride in aqueous solution.

METHOD

It was our desire to prepare an allergenically active product from whole pollen so that (1) all of the original fractions in the pollen grain would be incorporated into the extract and (2) it would be in a form which would lend itself to simple standardization and easy administration even to very sensitive persons. After many trials, an extraction fluid consisting of one part pyridine and one part 0.3 per cent sodium bicarbonate solution was chosen. Pyridine is a weak tertiary base and an important solvent because of its solvent action on many organic substances. The extract was prepared as follows: Five grams of ragweed pollen was allowed to extract with 100 ml. of the pyridine-bicarbonate solution for twenty-four hours. The extract was filtered off and

then Seitz-filtered for sterilization. Under sterile conditions, to one part of sterile distilled water was added one part of the 5 per cent pyridine-bicarbonate ragweed extract, and then one part of a solution of sterile 2 per cent potassium aluminum sulfate in ¼ N sulfuric acid was added slowly with mixing causing precipitation of an allergenically active water-insoluble whole ragweed complex. The precipitate and solution were allowed to stand overnight at 5° C. Then the mixture was centrifuged and the supernatant fluid was discarded. The residue was then washed three times with large quantities of sterile saline solution, and sterile glass beads were used to separate the particles of the precipitate and facilitate washing. The final volume of this suspension was made up to a volume equal to the initial volume of the 5 per cent pyridine-bicarbonate ragweed extract used.

TABLE II. POSSIBLE STANDARDS ON THE SUSPENSIONS OF WATER-INSOLUBLE WHOLE RAGWEED POLLEN COMPLEX

Protein nitrogen unit: Mg. protein nitrogen × 100,000	23,000
Total nitrogen unit: Mg. total nitrogen × 100,000	23,300
Total weight unit: Mg. dry weight of whole ragweed complex × 2,000	24,000
Noon or pollen unit: Mg. pollen extracted per milliliter × 1,000	50,000
Weight/volume standard: Grams of pollen extracted per milliliter	1:20

An aliquot of the suspension was taken for a phosphotungstic-acid-precipitable nitrogen (protein nitrogen) determination as described by Cooke and Stull.[20] Another aliquot was carefully measured into a weighed fritted glass filter, filtered with the aid of suction, and dried at 37° C. for twenty-four hours, which gave a constant weight at this temperature and time interval. Weight and nitrogen determinations on different lots of the 5 per cent suspension show a constant proportion between the nitrogen and weight values. Stull and Cooke converted milligrams of phosphotungstic-acid-precipitable nitrogen into the protein nitrogen unit system by multiplication by 100,000. We propose to multiply our total dry weight by 2,000 to convert to the approximate protein nitrogen unit value. For example, the dry weight of one lot was found to be 12 mg. per milliliter and the protein nitrogen was 0.233 mg. If, as proposed above, 12 is multiplied by 2,000, a figure of 24,000 units is reached and 23,300 units were actually found by nitrogen determination (Table II).

ABSORPTION TIME

The difference in the rate of absorption of the ordinary aqueous ragweed extract and the new ragweed complex suspension was determined by passive transfer tests which were performed on six suitable normal nonsensitive

adults, and the time interval and degree of reaction at the passive transfer sites were noted. It was found that there was a definite decrease (threefold or more) in the rate of absorption of the ragweed complex suspension over the absorption of an ordinary ragweed extract (Table III).

TABLE III. TIME AND DEGREE OF REACTION UPON PASSIVELY SENSITIZED SITE AFTER INJECTION OF RAGWEED ANTIGEN IN OPPOSITE ARM

	AQUEOUS EXTRACTS, 5,000 UNITS		SUSPENSION OF WHOLE RAGWEED COMPLEX, 5,000 UNITS	
CASE NO.	TIME INTERVAL (MINUTES)	REACTION	TIME INTERVAL (MINUTES)	REACTION
1	30	Marked	90	Moderate
2	45	Moderate	140	Moderate
3	30	Marked	110	Moderate
4	45	Moderate	130	Slight
5	25	Marked	90	Moderate
6	22	Marked	90	Marked

Slight = moderate erythema; moderate = small, round wheal with an area of erythema; marked = large wheal, irregular, with pseudopods and an area of erythema.

In mid-August of 1955, twenty highly sensitive adult hay fever patients were selected for coseasonal treatment on an experimental basis by injection with the new whole ragweed complex suspension. These patients were chosen because of the fact that, due to their high degree of sensitiveness, they were unable to undergo satisfactory treatment or reach maximum* or near maximum dosages with the aqueous ragweed extract. Each patient was tested intracutaneously with the ordinary aqueous ragweed pollen extract as well as with the suspension of whole ragweed complex in dilutions of 10, 100, and 500 protein nitrogen units. These tests showed no reactions or only slight reactions in eighteen of the twenty patients tested with the new suspension of whole ragweed complex, whereas, because of highly sensitive skin (dermographism), in the remaining two patients reactions were identical to those shown with the regular aqueous ragweed extracts. The patients were classified as AA, A, or B, according to the degree of reactivity as indicated by testing with the aqueous ragweed extract; AA was the most sensitive and B the least sensitive of the group. This classification served as a guide to the average dosage schedule suitable for prophylactic treatment, which was then begun by using the pyridine alum-precipitated whole ragweed complex. Eight patients were in Class AA, seven were in Class A, and five were in Class B. Class C patients were not chosen for treatment with the new ragweed complex suspension since they present no problem when treated with the regular aqueous extracts. They demonstrate a mild degree of reactivity, as indicated by intracutaneous tests with aqueous pollen extracts, and are able to reach maximum or near maximum dosages with the aqueous extract. Since the mixture was new and untried, the standard schedule of dosages usually employed with the aqueous ragweed pollen extracts was followed (Table IV). After several injections had been administered at weekly intervals without discomfort to

*Maximum dosage is the dosage which, if exceeded, is likely to induce a marked local or constitutional reaction.

TABLE IV. PROPHYLACTIC TREATMENT; AVERAGE DOSAGE FOR VARIOUS CLASSES GIVEN IN PROTEIN NITROGEN UNITS (STULL-COOKE)

		CLASS AA (1 UNIT = MARKED; 10 UNITS = MARKED PLUS) (UNITS)	CLASS A (10 UNITS = MARKED; 100 UNITS = MARKED PLUS) (UNITS)	CLASS B (100 UNITS = MARKED; 500 UNITS = MARKED PLUS) (UNITS)	CLASS C (100 UNITS = MODERATE; 500 UNITS = MARKED) (UNITS)
Test	Day	0	0	0	5
Inj.	1	2	5	10	10
Inj.	2	5	10	20	20
Inj.	3	10	20	40	40
Inj.	4	20	30	70	70
Inj.	5	30	40	100	100
Inj.	6	40	60	200	200
Inj.	7	50*	80	300	400
Inj.	8	--	100	400	700
Inj.	9	--	150	600	1,000
Inj.	10	--	200	800	1,250
Inj.	11	--	300	1,000	1,500
Inj.	12	--	400	1,250	2,000
Inj.	13	--	500*	1,500	2,500
Inj.	14	--	---	1,750	3,000
Inj.	15	--	---	2,000	3,500
Inj.	16	--	---	2,250	4,000
Inj.	17	--	---	2,500*	5,000*

*This dose continued at four- to seven-day intervals during the season.

any patient and with no reactions or only small local reactions resulting, dosages greater than those which ordinarily would be administered with the aqueous ragweed extract were given. Antihistaminic drugs were administered in addition to the immunization treatment for symptomatic relief. A significant observation during the coseasonal treatment with the new suspension was a notable absence of itching of the eyes, which is a predominant symptom in pollen-sensitive persons. No local irritation, dermatitis, or toxic effect was noticed with the use of the new extract. The local reactions from injections with the suspension attained their maximum size in about one-half to four hours after injection. Two of the twenty patients experienced untoward reactions in this initial trial with the suspension. These reactions occurred in extremely sensitive patients and were mild and delayed requiring no medication for relief. One patient (Table V, Case 17) had slight tightness of the chest and itching of the palms one hour after injection of 70 units. The second patient (Table V, Case 15) had a marked local reaction with itching of the hands one hour after injection of 200 units. These reactions occurred because the dosages were excessively increased during this study. In one patient (Table V, Case 20) an injection of 20 units of the aqueous ragweed extract was substituted one week after the same amount of the suspension had been injected without ill effect, and a severe local reaction involving the entire arm occurred. Subsequently, 30 units of the suspension were injected with no local reaction resulting.

In 1956 thirty hay fever patients were selected for treatment with the suspension since they were unable to undergo satisfactory treatment with the aqueous ragweed extracts. This group included patients who demonstrated

TABLE V. DOSAGE SCHEDULE (1955)*
(GIVEN IN PROTEIN NITROGEN UNITS)

CASE NO.	INJECTION															MAXIMUM DOSE† AQUEOUS EXTRACT (PREVIOUS YEARS)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
									Class B							
1	25	50	100	200	400	700[6]										None‡
2	25	50	100	200	400	700	1,000	1,500[1]	2,000[1]	2,500[3]	3,000[3]	4,000[3]	5,000[3]			1,500
3	25	50	100	200	400	700	1,000	1,500	2,000	2,500	3,000[1]	4,000[3]	5,000[3]	6,000[3]		2,500
4	25	50	100	200	400	700	1,000	1,500	2,000	2,500[1]	3,000[1]	4,000[3]	5,000[3]			2,000
5	10	20	40	70	100	200	400	700	1,000	1,500	2,000[1]	3,000[3]	4,000[3]			2,000
									Class A							
6	10	20	40	70	100	200	400	700	1,000[6]							None
7	5	10	20	30	40	60	80	100	200	300	400	600	800	1,000[6]		500
8	10	20	40	70	100	200	400	700[1]	1,000[2, 6]							1,000
9	10	20	40	70	100	200	400	700	1,000							400
10	5	10	20	30	40	60	70	100	150	300	500					60
11	5	10	20	30	40	60	80	100	200	300	400	600	800	1,000	1,500[2]	200
12	10	20	30	40	60	80	100	200	400							30
									Class AA							
13	5	10	20	30	40	70	100	150	200	250	300	400	600	800	1,000[1]	100
14	5	10	20	30	40	70	100	200	300[4]							10
15	5	10	20	30	40	60	80	100	200[4, 5]	100[6]						None
16	2	5	10	20	30	40[4, 6]										30
17	2	4	7	10	15	20	30	40	70[5]	40						8
18	5	10	20	30	40	50[6]										None
19	2	3	4	5	6	7	8	9	10[4]							2
20	5	10	20	20[7]	30	40	50	60	70[6]							None

*Injections were given at weekly intervals, unless otherwise noted. Exceptions and reactions are symbolized as follows:
[1]Given after interval of two weeks.
[2]Given after interval of three weeks.
[3]Given after interval of four weeks.
[4]Marked local reaction.
[5]Mild systemic reaction.
[6]Patient did not return for further treatment.
[7]Aqueous extract given and marked local reaction occurred.
†Maximum dosage is the dosage which, if exceeded, is likely to induce a marked local or constitutional reaction.
‡No previous hay fever treatment.

a high degree of sensitiveness, as shown by intracutaneous testing, or who in previous years demonstrated repeated marked local or constitutional reactions and therefore could not receive maximum dosages of the aqueous ragweed extracts. The patients were classified by intracutaneous testing with aqueous ragweed extracts; ten patients were in Class AA, fourteen were in Class A, and six were in Class B. There were no Class C cases. Since treatment with the suspension in 1955 had made it apparent that a schedule with a decreased number of injections was possible, the schedule employed with the aqueous pollen extracts was not followed. In general, the schedule that was successfully employed in our clinic with the use of slowly absorbed gelatin pollen extracts[15] was adopted (Table VI).

TABLE VI. PROPHYLACTIC TREATMENT, AVERAGE DOSAGE FOR VARIOUS CLASSES SUSPENSION OF WHOLE RAGWEED COMPLEX GIVEN IN PROTEIN NITROGEN UNITS*

INJECTION	CLASS AA (UNITS)	CLASS A (UNITS)	CLASS B (UNITS)	CLASS C (UNITS)
1	10	25	25	50
2	20	50	50	150
3	30	100	100	300
4	40	200	200	700
5	60	300	400	1,000
6	80	500	700	2,000
7	100	700	1,000	3,500
8	200	1,000	1,500	5,000
9	300	1,500	2,000	7,000
10	400	2,000	2,500	8,000
11	600	2.500	3,000	10,000
12	800	3.000	4,000	
13	1,000	3,500	5,000	
14		4,000	6,000	
15		5,000	7,000	
16			8,000	
17			9,000	
18			10.000	

*1 unit = 0.00001 mg. protein nitrogen.

The Class AA patient received 10 units as an initial injection, and the Class A or B patient received 25 units. The schedule was often modified to suit the individual's tolerance for the suspension (Table VII). The Class B patient usually reached 2,500 units at the tenth injection with the suspension, as compared to at least seventeen injections with the aqueous extract and 5,000 units at the thirteenth injection of the suspension. One patient classified as B reached 10,000 units in eighteen injections. The Class A patient usually reached 2,000 units at the tenth injection and an approximate average of 3,000 units in twelve injections. The extremely sensitive Class AA patient reached an average of 600 units at the eleventh injection. It is difficult to achieve such high dosages in very sensitive patients with the aqueous extract. Some of the patients might possibly have taken greater doses, but no attempt was made to increase the dosage further as the ragweed concentration was often twice the amount attained with the aqueous extracts. Another important advantage is the ability to administer the suspension in the prescribed doses

at intervals greater than one week, in some cases as long as four weeks. In Fig. 1 a comparison is made of the average dosage schedule when the suspension is used and the schedule followed with the standard aqueous extracts.

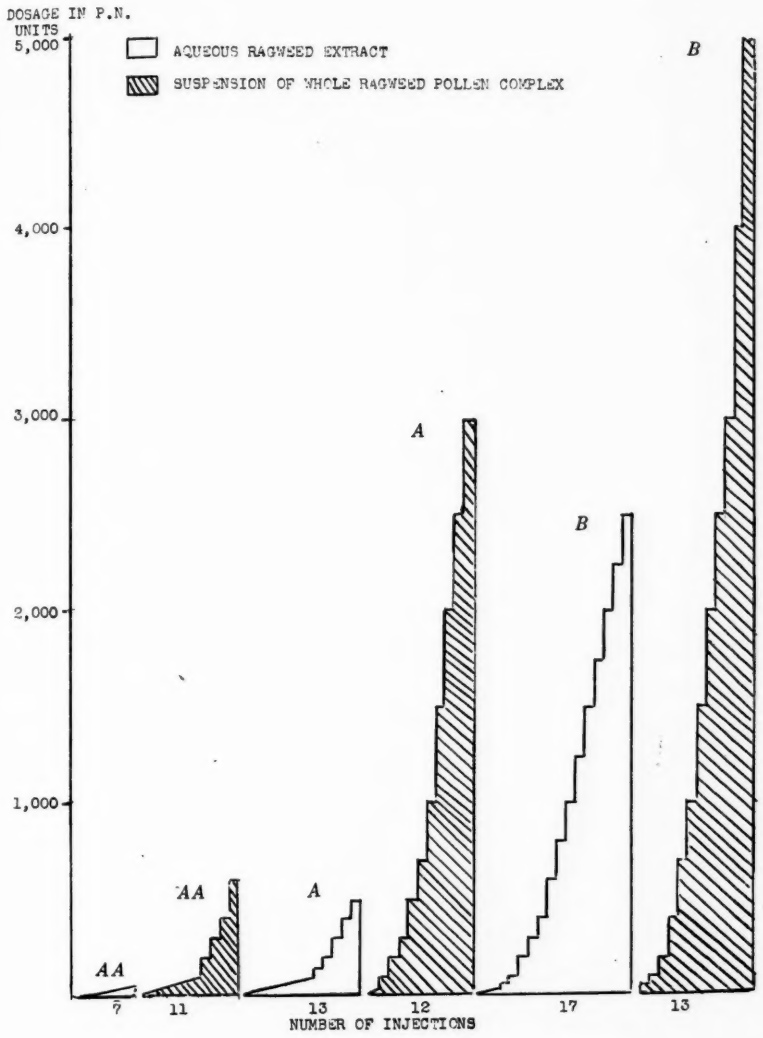

Fig. 1.

Twenty-six, or 86.7 per cent, of the thirty patients who received preseasonal treatment with the whole ragweed complex obtained satisfactory relief, against twenty-three, or 76.7 per cent, in a control group of thirty less sensitive patients treated with the standard aqueous extracts and with antihistaminic drugs and other medications (Table VIII). These results were determined by a study of the daily symptoms recorded by each patient. An

TABLE VII. DOSAGE SCHEDULE (1956)*
(GIVEN IN PROTEIN NITROGEN UNITS)

CASE NO.	INJECTION 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	MAXIMUM DOSE† AQUEOUS EXTRACT (PREVIOUS YEARS)
										Class B									
1	25	50	100	200	400	700	1,000[1]	1,500[1]	2,000[1]	3,000[3]	4,000[3]	5,000[3]							2,000
2	25	50	100	200	400	700	1,000	1,500[1]	2,000[3]	3,000[3]	4,000[3]	5,000[3]							2,000
3	25	50	100	200	400	700	1,000	1,500	2,000	2,500	3,000[3]	3,500[3]	4,000[3]	5,000[3]					None‡
4	25	50	100	200	400	700	1,000	1,500	2,000	2,500	3,000	4,000	5,000						2,000
5	25	50	100	200	400	700	1,000	1,500	2,000	2,500[1]	3,000[1]	4,000[1]	5,000[3]						2,000
6	25	50	100	200	400	700	1,000	1,500	2,000	2,500	3,000	4,000	5,000[2]	6,000[3]	7,000[3]	8,000[3]	9,000[3]	10,000[3]	5,000
										Class A									
7	25	50	100	200[4]	100	200	300	500	700	1,000	1,500	2,000							1,000
8	25	50	100	200	400	700	1,000	1,500	2,000	2,500	3,000[1]	3,500[1]	4,000[1]	5,000[3]					None
9	25	50	100	200	300	400	700	1,000	1,500	2,000	2,500	3,000							500
10	25	50	100	200	300	500	700	1,000	1,500	2,000	2,500	3,000	3,500						400
11	25	50	100	200	300[1]	400	700	1,000	1,500	2,000	2,500	3,000[1]	4,000[1]	5,000[3]					1,250
12	25	50	100	200	300	500	700	1,000	1,500	2,000	2,500[1]	3,000[1]	3,500[1]						None
13	25	50	100	200	300[4]	300	400	700	1,000	1,500	2,000	2,500[1]	3,000[1]	3,500[1]	4,000[3]	5,000[3]			1,000
14	25	50	100	200	400	700	1,000	1,500	2,000	2,500	3,000	3,500	4,000[1]	5,000[1]					600
15	25	50	100	200[1]	300	400	700	1,000	1,500	2,000	2,500	3,000							400
16	25	50	100	200	400	700	1,000	1,500	2,000	2,500	3,000								700
17	25	50	100	200	300	500	700	1,000	1,500	2,000									250
18	25	50	100	200	300	500	700	1,000	1,500	2,000[4]	2,000[4]	2,500							1,250
19	25	50	100	200	300	500	700	1,000[4]											350
20	25	50	100	200[4]	200	300	400[4]	300											80

								Class AA							
21	10	20	30	40	70	100	200	300	400	600	800	1,000	1,500[5]	1,000	200
22	10	20	30	40	60	80	100	200	300	400	600	800	1,000		100
23	10	20	30	40	60	80	100	200	300	400	600	800	1,000		100
24	10	20	30	40	60	80	100	200	300	400	500	600	700[4]		100
25	10	20	30	40	60	80	100	200	300	400	600	800			150
26	10	20	30	40	60	80	100	200[5]							20
27	10	20	30	40	60	80	100	200	300	400					None
28	10	20	30	40	60	80	100	200	300	400	600				200
29	10	20	30	40	60	80	100	200[4]	100						70
30	10	20	30	40	60	80[5]	60								10

*Injections were given at weekly intervals unless otherwise noted. Exceptions and reactions are symbolized as follows:

[1]Given after interval of two weeks.

[2]Given after interval of three weeks.

[3]Given after interval of four weeks.

[4]Marked local reaction.

[5]Mild systemic reaction.

†Maximum dosage is the dosage which, if exceeded, is likely to induce a marked local of constitutional reaction.

‡No previous hay fever treatment.

evaluation of the results of the treatment of the two groups of hay fever patients was feasible, since the less sensitive group treated with the standard aqueous extracts accepted high dosages which in many instances approximated the maximum dosages (unit per unit of protein nitrogen) attained by the group of more sensitive hay fever patients treated with the whole pollen suspension. For example, the least sensitive Class C patient usually reached 5,000 units when treated with the aqueous extracts, and this same dosage of 5,000 units was attained by the more sensitive Class B patient treated with the whole pollen suspension.

TABLE VIII. RESULTS OF PRESEASONAL TREATMENT IN 1956

TREATMENT	NO. OF PATIENTS	NO OR SLIGHT HAY FEVER		NO SEVERE HAY FEVER		FIVE OR LESS SEVERE DAYS		SEVERE HAY FEVER		TOTAL EFFECTIVE RELIEF	
		NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT
Suspension of whole ragweed complex	30	14	46.7	8	26.7	4	13.3	4	13.3	26	86.7
Aqueous ragweed extract*	30	8	26.7	6	20.0	9	30.0	7	23.3	23	76.7

*This group of patients received antihistaminics when hay fever symptoms occurred.

Again, as noted during the hay fever season of 1955, there was a conspicuous absence of itching of the eyes in the pollen-sensitive patients treated with the whole pollen complex suspension; this symptom had given them great discomfort during previous pollen seasons. Itching of the eyes was a major complaint of many patients treated with the defatted pollen.

The local reactions from injections with the ragweed complex suspension attained their maximum size in about one-half to four hours after injection. No local irritation or induration at the sites of the injection occurred. In only three highly sensitive Class AA patients did mild systemic reactions, such as itching of the palms, stuffiness of the nose, sneezing, cough, or slight tightness in the chest, appear following treatment (Table VII, Cases 21, 26, and 30). These reactions were not severe enough to require medication.

In 1957 twenty-eight highly sensitive hay fever patients were treated preseasonally with the suspension. Eight patients were evaluated as Class AA, seventeen as Class A, and three as Class B. In treating these patients, we generally followed the schedule with a decreased number of injections employed in 1956 (Table VI). The Class AA patients received 10 units as an initial injection, and the Class A and Class B patients received 25 units. The number of injections required to reach maximum dosages for these highly sensitive patients was almost identical with the number of dosages of the suspension administered to hay fever patients in 1956, and the ragweed concentration was often twice the amount attained with the aqueous extracts (Table IX and Fig. 1).

Ten other highly ragweed-sensitive patients who had reached maximum or near maximum dosages of the suspension at the end of the hay fever season in 1956 continued their treatment throughout 1957, receiving maximum dosages ranging from 2,500 to 5,000 units (Table IX, Cases 29 to 38). In previous years these highly pollen-sensitive patients were unable to undergo the perennial form of treatment, since they could not attain maximum dosages with aqueous extracts. It was noted that with the suspension a change from the old to the new suspension could be made without lessening the dosage and with no untoward reactions.

Twenty-four, or 85.7 per cent, of the twenty-eight patients treated preseasonally with the suspension obtained satisfactory relief as compared with twenty-one, or 75 per cent, in a control group of twenty-eight less sensitive patients treated with aqueous extracts and antihistaminic drugs (Table X).

Of the ten hay fever patients treated by the perennial method with injections of maximum doses of the suspension, nine, or 90 per cent, experienced effective relief, as compared with seven, or 70 per cent, in a control group of ten patients treated with aqueous pollen extracts.

No local irritation, induration, or dermatitis was noted at the sites of the injections. Again, as noted in the two previous years, there was a remarkable absence of itching of the eyes in the pollen-sensitive patients treated with the suspension.

One extremely sensitive patient (Table IX, Case 28) complained of slight tightness in the chest one-half hour after injection of 40 units of the suspension. This mild systemic reaction disappeared within ten minutes without any medication. The dosage was subsequently reduced to 30 units and then increased to a maximum of 60 units with no ill effects. Another patient (Table IX, Case 10) who had received 2,500 units of the suspension with no untoward effects was accidentally given 2,500 units of the aqueous ragweed extract. There followed immediately a severe local and systemic reaction which required repeated epinephrine injections, ephedrine compounds, and antihistaminics. The patient experienced severe hay fever symptoms, generalized urticaria, and asthma which persisted for three days and required urgent medical treatment. Two weeks later 2,500 units of the suspension was injected with safety, and these injections were repeated each month.

DISCUSSION

The treatment of hay fever and the standardization of pollen extracts do not fulfill all requirements, probably due to the type of extract used. Since it was assumed that the oil fraction was not responsible for hay fever symptoms, practically all pollen extracts for therapeutic or experimental use are prepared by first defatting and dehydrating the pollen with ether. Rockwell[18] suggested that the active component of pollen was a flavonol-carbohydrate-proteose complex. When pollen was treated with ether to eliminate the oil prior to aqueous extractions, such a complex would be broken, due (1) to removal of the flavonol and (2) to some denaturization through dehydration and would thus lead to a changed antigenic component in aqueous solution.

TABLE IX. DOSAGE SCHEDULE (1957)*
(GIVEN IN PROTEIN NITROGEN UNITS)

CASE NO.	INJECTION 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	MAXIMUM DOSAGE† AQUEOUS EXTRACT (PREVIOUS YEARS)
									Class B									
1	25	50	100	200	400	700	1,000	1,500	2,000	2,500	3,000	4,000[1]	5,000[1]	6,000[3]	7,000[3]	8,000[3]	10,000[3]	3,000
2	25	50	100	200	400[4]	600[4]	800	1,000	1,500	2,000	2,500[4]	2,500[3]	3,000[3]	4,000[3]				600
3	25	50	100	200	400	700	1,000	1,500	2,000	2,500								1,000
									Class A									
4	25	50	100	200	400	700	1,000	1,500	2,000[1]	2,500[1]	3,000[1]							1,000
5	25	50	100	200	400	700	1,000	1,500	2,000[1]	2,500[1]	3,000[1]							None‡
6	25	50	100	200	400	600	800	1,000	1 500	2,000	2,500	3,000[4]						None
7	25	50	100	200	400	700	1,000	1,500	2,000	2,500								100
8	25	50	100	200	400	700	1,000	1,500[1]	2,000[1]	2,500[1]								200
9	25	50[4]	70	100	200	400	700	1,000	1,500	2,000	2,500							200
10	25	50	100	200	400	700	1,000	1,500	2,000	2,500	2,500[7]	2 500[1]	2,500[3]					None
11	25	50	100	200	300[4]	300	400	600	800	1,000	1,500[1]	2,000[1]						None
12	25	50	100	200	400	700[1]	1,000[1]	1,500[1]	2,000[4]	2,000[4]								250
13	25	50	100	200	400	700	1,000	1,500[1]	2,000[1]									None
14	25	50	100	200	400	700	1,000	1,500	2,000									700
15	25	50	100	200	400	700	1,000	1,500	2,000									None
16	25	50	100	200	400	700	1,000	1,500										60
17	25	50	100	200	300[4]	300	400	500	700	1,000								150
18	25	50	100	200	300	400	700	1,000[4]	1,000									None
19	25	50	100	200	400	700	1,000	1,500[5]	1,000									None
20	25	50	100	200	400	700	1,000[5]	1,000[4]										350
									Class AA									
21	10	20	40	70	100	200	300[2]	400	500	600	800	1,000[1]						150
22	10	20	40	70	100	200	300	400	500	700								200
23	10	20	40	70	100	200	300	400										80
24	10	20	40	70	100	200[4]	300[4]	400[5]										None
25	10	20	40	70	100	200	300	400										None
26	10	20	40	70	100[4]	150[4]	200											10
27	10	20	30	50	70[4]													5
28	10[5]	5	10	20	30	40[6]	30	40	60[4]									10

	Perennial Cases												
29	5,000	5,000[3]	5,000[3]	5,000[3]	5,000[3]	5,000[3]	5,000[3]	5,000[3]	5,000[3]	5,000[3]	5,000[3]	5,000[3]	600
30	5,000	5,000[3]	5,000[3]	5,000[3]	5,000[3]	5,000[3]	5,000[3]	5,000[3]	5,000[3]	5,000[3]	5,000[3]	5,000[3]	800
31	5,000	5,000[3]	5,000[3]	5,000[3]	5,000[3]	5,000[3]	5,000[3]	5,000[3]	5,000[3]	5,000[3]	5,000[3]	5,000[3]	2,000
32	5,000	5,000[3]	5,000[3]	5,000[3]	5,000[3]	5,000[3]	5,000[3]	5,000[3]	5,000[3]	5,000[3]	5,000[3]	5,000[3]	2,500
33	5,000	5,000[3]	5,000[3]	5,000[3]	5,000[3]	5,000[3]	5,000[3]	5,000[3]	5,000[3]	5,000[3]	5,000[3]	5,000[3]	1,000
34	4,000	4,000[3]	4,000[3]	4,000[3]	4,000[3]	4,000[3]	4,000[3]	4,000[3]	4,000[3]	4,000[3]	4,000[3]	4,000[3]	800
35	3,500	3,500[3]	3,500[3]	3,500[3]	3,500[3]	3,500[3]	3,500[3]	3,500[3]	3,500[3]	3,500[3]	3,500[3]	3,500[3]	1,000
36	2,500	2,500[3]	2,500[3]	2,500[3]	2,500[3]	2,500[3]	2,500[3]	2,500[3]	2,500[3]	2,500[3]	2,500[3]	2,500[3]	1,250
37	2,500	2,500[3]	2,500[3]	2,500[3]	2,500[3]	2,500[3]	2,500[3]	2,500[3]	2,500[3]	2,500[3]	2,500[3]	2,500[3]	400
38	2,500	2,500[3]	2,500[3]	2,500[3]	2,500[3]	2,500[3]	2,500[3]	2,500[3]	2,500[3]	2,500[3]	2,500[3]	2,500[3]	1,000

*Injections were given at weekly intervals, unless otherwise noted. Exceptions and reactions are symbolized as follows:
[1]Given after interval of two weeks.
[2]Given after interval of three weeks.
[3]Given after interval of four weeks.
[4]Moderate local reaction.
[5]Marked local reaction.
[6]Mild systemic reaction.
[7]Severe systemic reaction occurred when aqueous extract was given instead of suspension of whole pollen complex.
†Maximum dosage is the dosage which, if exceeded, is likely to induce a marked local or constitutional reaction.
‡No previous hay fever treatment.

TABLE X. RESULTS OF PRESEASONAL TREATMENT IN 1957

TREATMENT	NO. OF PATIENTS	NO OR SLIGHT HAY FEVER		NO SEVERE HAY FEVER		FIVE OR LESS SEVERE DAYS		SEVERE HAY FEVER		TOTAL EFFECTIVE RELIEF	
		NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT
Whole ragweed complex suspension	28	14	50.0	7	25.0	3	10.7	4	14.3	24	85.7
Aqueous ragweed extract*	28	8	28.6	5	17.8	8	28.6	7	25.0	21	75.0

*This group of patients received antihistaminics when hay fever symptoms occurred.

A suspension of a new water-insoluble whole ragweed pollen complex has been prepared. This preparation is in a form which lends itself to easy administration and slow absorption. It can be standardized by a weight method which takes into account all antigenic fractions present. A direct correlation between the nitrogen value and weight of the complex is obtained. The initial extraction is made upon whole pollen which has not been previously treated with an organic solvent, such as ether for defatting purposes. Since this paper was read, Cohen and Michelini[21] have reported that there is a qualitative and quantitative antigenic superiority in the whole ragweed pollen grain which could not be duplicated with immunizing procedures using preparations of pollen extracts. They also showed that ragweed pollen oil contains one antigenic fraction. The extracting fluid consisting of one part pyridine and one part 0.3 per cent sodium bicarbonate was chosen for the initial step in this preparation, so that the oil fraction in the pollen grain could be readily dissolved with the proteinaceous fraction. Experimental data on the antigenic components of this alum-precipitated pyridine ragweed complex will be reported upon at a later date. Even though this suspension of whole ragweed complex was not suitable for intracutaneous skin testing, it was felt that this was due to its slow absorption since constitutional reactions did occur in sensitive persons when the dosage was unduly increased.

Seventy-eight ragweed hay fever patients, highly sensitive to pollen, were treated with the suspension during a three-year period (1955, 1956, and 1957). The patients selected for treatment with the suspension consisted of those who were unable to undergo satisfactory treatment with aqueous extracts because of their marked degree of sensitiveness. A dosage schedule with a reduced number of injections was generally followed, since a greater tolerance for this new suspension was noted. Longer intervals between dosages were found feasible. The local reactions from injections with the suspension attained their maximum size in about one-half to four hours after injection. While this new suspension seems to be a suitable material for slow absorption, it does not entirely eliminate constitutional reactions. When untoward reactions do occur, they are usually mild and delayed, occurring one to four hours after treatment when the patient is no longer within reach of his physician. Some of the patients might possibly have taken much greater top doses, but no attempt was made to increase the dosage as the ragweed concentration was often twice the amount attained with the aqueous extracts. Occasionally, there may be an extremely sensitive patient who will not tolerate more than 2 or 3 units of the aqueous ragweed extract. Such a sensitive person should be treated conservatively, even with the suspension, and the dosages should not be excessively increased. Although Milford[6] and Moore and associates[7] showed that there was an allergenically active component in pollen oil, Cooke and Stull[20] stated that the complete removal of the ether-soluble material in pollen was indicated in order to avoid the danger of producing dermatitis in extremely susceptible patients and to facilitate the extraction of the active material. In our series of seventy-eight highly sensitive patients no local induration, irritation dermatitis, or toxic effects were noted.

Ten highly ragweed-sensitive patients who had completed a course of preseasonal treatments with the suspension continued to receive their maximum dosages at monthly intervals thereafter. These patients could not attain such high dosages with aqueous extracts. It was possible to change directly from the old suspension of whole ragweed pollen complex to a new suspension without lessening the dosage.

Treatment with the suspension seems to have given more effective relief than the standard aqueous pollen extracts. A significant observation following treatment with the new suspension of whole ragweed complex was a notable absence of itching of the eyes, which is a predominant and discomforting symptom in hay fever patients. The symptom of itching eyes may be caused by the oil fraction of pollen; therefore, the absence of this symptom following treatment with this special type of whole ragweed pollen product may possibly be explained on the basis of successful immunization with this whole pollen complex suspension which also contains the oil fraction.

SUMMARY

1. A suspension of a new water-insoluble whole ragweed pollen complex, believed to be more representative of all the allergenic components as they appear in the pollen and in a form which lends itself to slow absorption, was prepared.

2. The suspension can be standardized by a weight method which takes into account all antigenic fractions present.

3. A direct correlation between the nitrogen value and weight of the alum-precipitated pyridine whole ragweed complex is obtained.

4. A series of seventy-eight adult patients, highly sensitive to ragweed pollen, were treated with the suspension. Ten patients of this group continued their treatment on a monthly basis after having completed a course of preseasonal injections.

5. A greater tolerance for the suspension was noted (often twice the amount attained with aqueous extract was given with safety and often with one-half the number of injections).

6. Constitutional reactions were mild, delayed, and infrequent.

7. Excellent clinical relief was obtained following treatment of extremely sensitive patients treated with the suspension.

8. There was a notable absence of itching of the eyes in the hay fever patients treated with the suspension.

9. The symptom of itching eyes may be caused by the oil fraction of pollen, and the observed absence of this symptom following treatment may have been the result of successful immunization with the whole pollen extract which also contained the oil fraction.

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AN IMPROVED VOLUMETRIC IMPINGER FOR POLLEN COUNTING

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THE first studies of atmospheric pollen concentrations were conducted by Scheppegrel[1] in 1917. He attempted to derive a formula for converting the number of pollens on a square centimeter of slide to a volumetric equivalent by applying Stokes's law for small falling bodies.[2] Using this formula, Scheppegrel arrived at a factor of 1.8 for converting the number of ragweed pollens on a square centimeter of slide to a cubic yard equivalent. This remained the basis for counting and converting atmospheric equivalents until 1937, when Cocke[3, 4] pointed out some difficulties in these formulas. In the first place, Stokes's law pertained to smooth, spherical bodies in still air; pollens are, for the most part, spiculated and aspherical, and the air is usually in motion. Second, it is necessary to determine accurately the rate of fall, the specific gravity, and the size of each pollen before one is able to apply Stokes's law. One would then obtain a separate factor for each pollen. Third, Cocke pointed out that Scheppegrel had erred in his original calculations and had used the diameter and not the radius of the pollen in calculating his factor for ragweed.

Dahl and Ellis[5] reported on pollen concentrations of the atmosphere in 1942. Durham[6-12] did monumental work in conducting pollen surveys in this country and in developing a standard method of pollen counting. The major portion of this work was done by the gravity-slide method, utilizing pollen shelters of varying construction. The advantages of this method were its simplicity and the rapidity with which one could obtain the desired information. No further refinements were made in the procedure of pollen counting until 1946 when Durham,[11] dissatisfied with Scheppegrel's formula, ran parallel gravity-slide and volumetric studies. This work formed the basis for the adoption of standard apparatus, counting procedure, and conversion factor by the Pollen Survey Committee of the American Academy of Allergy[13] in 1947. The conversion factor adopted for ragweed was 3.6, just double the previous one. At that time it was the feeling of the members of the Committee that the gravimetric method, even with the revised conversion factor, was unsatisfactory and that a volumetric method, if available, would be more accurate and preferable. Among the factors which prevent an accurate conversion from the gravity slide to the volumetric equivalent are such variables as the location of the shelter, wind velocity, wind direction, wind direction in relation to the source of the pollen, and convection and deflection currents.

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The standard gravity method of the American Academy of Allergy had the advantage of being simple, inexpensive, and devoid of moving parts. Its widespread use had given us much valuable information concerning the distribution of pollen in this country. For the reasons previously cited, however, the gravity method cannot be converted to volumetric figures with any real degree of accuracy. In addition, one is incapable of determining either pollen concentrations for any specified period of the day or the exact amount of pollen that a patient might be inhaling in a given period of time.

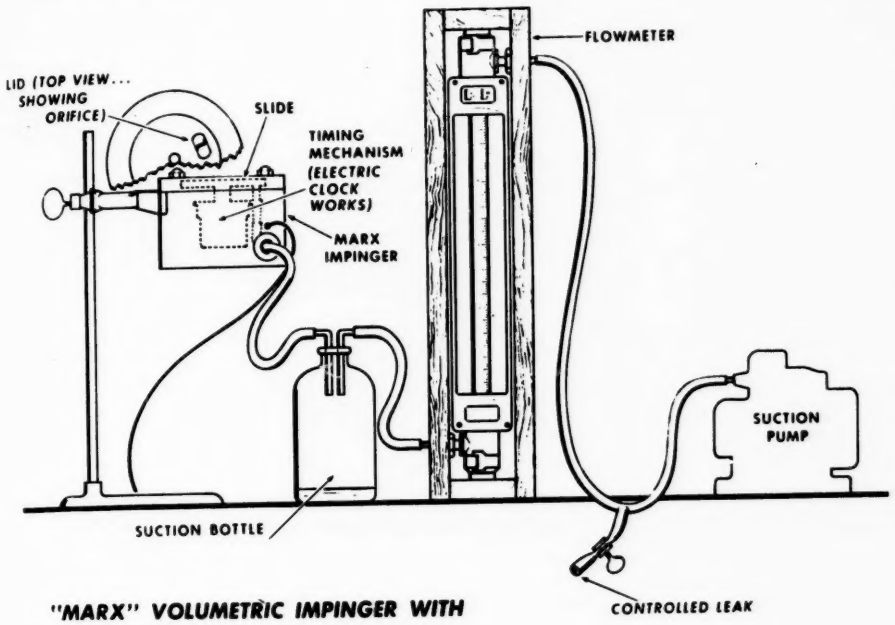
While most investigators were using the gravimetric method, several have attempted to use volumetric impingers. Duke[14] used a motor-driven hair drier which delivered a stream of air at constant velocity. This device, however, lacked volumetric control or calibration. Keitt and Jones[15] used a vacuum pump which pulled air through a nitrocellulose filter at the rate of 60 cubic feet per hour. Erdtman[16] tested the air over the Atlantic Ocean, using a vacuum cleaner and filter papers. In 1942, Hawes, Small, and Miller[17] devised an instrument which drew a known volume of air over a petrolatum-coated slide via a suction pump. The slide moved horizontally by means of a timing device at a rate that required frequent slide changes during a twenty-four-hour interval. This apparatus was used by Durham[9] in his parallel volumetric and gravimetric studies. Stenburg and Hall[18] devised a very ingenious volumetric sampler in which a given volume of air was impinged on the adhesive side of a moving transparent cellulose tape. After exposure, this was passed through a staining bath and the stained surface was covered with a facing piece of tape. This apparatus was used by Smith and Rooks[19] in their studies of the diurnal variation of pollen grains. Sheldon and Hewson,[20] at the University of Michigan, have used still another volumetric device in their studies of atmospheric pollution with aeroallergens. Their device consists of a millipore filter through which a known volume of air is drawn. In their preliminary studies they ran the apparatus for thirty minutes every four hours, which gave them six samples a day.

It is the purpose of this article to present a new and improved volumetric impinger, which is simple, needs attention only once in twenty-four hours, and can be run for weeks at a time or through an entire pollen season without any mechanical difficulty. In addition, we have calibrated this device to sample 10 liters of air per minute, which we believe is a significant volume since it approximates human tidal respiratory volume. The pollen count obtained by this method would then represent the approximate number of pollen grains inhaled by the average patient on the day in question.

The present apparatus was devised by Marx[21] in 1955, in connection with his work for a Master of Science Degree at Pennsylvania State University, and is a modification of an impinger used by Elder.[22] The apparatus (Fig. 1) consists of the impinger with its spindle or slide and timing mechanism, a flowmeter to measure the volume sampled, and a suction pump.* A moisture

*This apparatus was part of the scientific exhibit at the fourteenth annual meeting of the American Academy of Allergy in Philadelphia, Pennsylvania, Feb. 3 to 5, 1958.

trap is also pictured, but this is superfluous since no difficulty was presented by rainfall entering the machine during three years of experience. The Marx impinger is a cylinder of stainless steel, 6 inches in diameter, that contains an air-tight chamber which houses the spindle or slide. The lid is of clear plastic and is held to the body by means of six thumb screws. In order to create a tight seal, a rubber gasket and silicone grease are used between the



"MARX" VOLUMETRIC IMPINGER WITH FLOWMETER & SUCTION PUMP

Fig. 1.

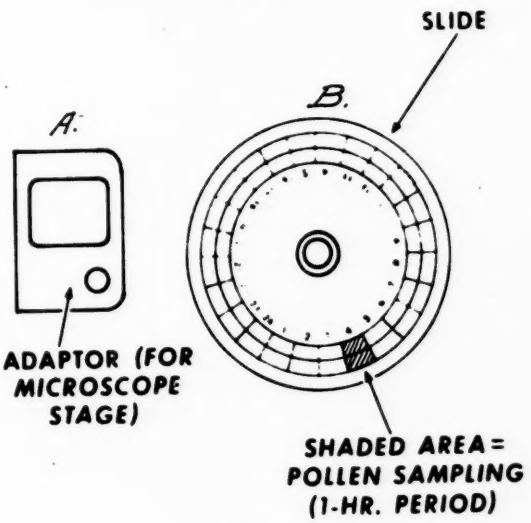


Fig. 2.

lid and the body. Within the apparatus itself is an electric timing mechanism that makes one complete revolution each twenty-four hours and upon which the spindle is mounted. The spindle is made of 7/32 inch clear plastic, 4 inches in diameter, and is engraved as shown in the drawing (Fig. 2, *B*). The circular markings are 10 mm. wide and are so described as to be directly beneath the 1 by 10 mm. orifice of the lid through which the air is drawn. The circle is divided into twenty-four equal parts, each representing an hour of the day. The shafts of the spindles are uniformly arranged to correspond to the hours of noon and midnight and to be interchangeable from a time standpoint. The lid and slide are so engineered that the drawn air will be impinged only on the engraved area of the slide, as pictured (Fig. 2, *B*). The efficiency of the impingement was demonstrated on numerous occasions by our inability to find pollen on any area other than those described by our slide markings and on petrolatum-coated slides that were placed beneath the spindles.

The suction source was a DeVilbiss portable suction-pressure pump. We found it capable of drawing up to 14 liters of air per minute, and it was efficient and trouble free when run continuously through an entire pollen season. It is important that this pump have its own electrical line to avoid interference by other equipment, which might tend to overload the circuit and thereby decrease the efficiency of the pump. A controlled leak, as shown in Fig. 1, was created in the circuit in order to maintain the flow of air at 10 liters per minute as measured by a Fischer-Porter flowmeter. For reasons stated previously, we chose this figure because it represents the average tidal volume and because a direct and meaningful pollen count is obtained. This is equal to 14,400 liters of air per twenty-four hours. This figure may be converted to cubic yard values by multiplying by the factor 1.308 by 10^{-3}, which gives its equivalent as 18.8 cubic yards of air per day.

The operation of the apparatus is simple. The circular plastic spindle is covered with a thin layer of petrolatum in the usual manner and then placed in the apparatus. Since all of our spindles are identical and time factors on each correspond to one another, it is a simple matter to remove the exposed spindle and to replace it with another at the same time each day. After making one complete revolution in twenty-four hours, the spindle is removed and taken to the laboratory where the actual counting is done. In order to make pollen identification clearer and easier, the slide was stained with Calberla's solution and then covered with a circular cover slip of 1/16 inch clear plastic which was fabricated especially for this purpose. We found that thinner plastic was unsatisfactory, for it tended to curl when in contact with the stain. At the present time we are experimenting with a specially prepared cover slip of glass, 1/32 inch thick, fabricated to fit the spindle. The entire assembly, spindle and cover slip, was then held on the microscope stage by mounting it on a small plastic adaptor as illustrated (Fig. 2, *A*). This permitted the operator to rotate the spindle manually through 360 degrees without deviating from the fixed circular field under scrutiny. The microscope employed was a binocular type with a 10× ocular and 10× objective. On days of

low pollen concentration, the entire ruled area for each hour was easily counted. On days of high concentration, however, this proved to be a monumental task. In an attempt to simplify the process, it became necessary to test the accuracy and validity of counting part of a single field and multiplying it by a factor, the product of which would be representative of the whole field. Over the period of observation twenty such areas have been counted. The average factor was 5.3. When subjected to statistical analysis, a standard deviation of 0.46 and a coefficient of variation of 8.6 per cent were obtained, showing a high degree of correlation. It must be made clear that this factor will vary from microscope to microscope, depending on the strength of its contained lenses and on the part of the ruled area which is represented by a single strip observation.

TABLE I

NUMBER OF POLLEN GRAINS ON A SINGLE STRIP	NUMBER OF POLLEN GRAINS FOR THE ENTIRE HOUR	FACTOR
12	62	5.12
33	201	6.00
27	141	5.22
6	33	5.50
7	36	5.14
17	103	6.05
19	71	3.72
17	75	4.41
15	78	5.20
5	25	5.00
26	144	5.53
37	196	5.60
16	100	5.55
10	49	4.90
23	124	5.42
6	33	5.33
34	186	5.47
29	154	5.31
7	38	5.43
3	16	5.33

Average factor = 5.3

DISCUSSION

Using the apparatus as described, we obtained counts for forty-five consecutive days during each of the 1956 and 1957 ragweed seasons. We encountered no difficulty with the impinger, pump, or flowmeter. After a short period of practice we found it no more difficult to count the pollen on our circular spindle than on a conventional gravity slide, although it was a bit more time-consuming because of the increased area covered. This apparatus has the added advantage of supplying information regarding the pollen concentration for each hourly period of the day. It is, therefore, useful in studying the diurnal pollen variations and the effects of wind, rainfall, and humidity on pollen counts. In addition, it could supply data that might correlate the relationship between clinical hay fever symptoms and pollen concentration. The apparatus may also be used to test the efficiency of air filtering and air conditioning equipment in removing pollen from the air. For such purposes, the timing mechanism may be set to turn the spindle at a more rapid rate.

The behavior of particulate substances in air varies with their size and shape and with the direction and velocity of the wind.[23] A perfect sampling device would have to be oriented to wind flow and be isokinetic, that is, draw air through the sampling device at the same rate as the wind velocity.[24] This would avoid disturbing or altering the arrangement of particulate agents suspended in the air. It would require a variable source of suction that would adjust to each change in wind velocity. A weather vane would compensate for alterations in wind direction. A perfect sampler of this type could accurately measure the pollen content of the air, but it could not estimate the average daily exposure by the human being, for the respiratory apparatus is not a perfect sampling device. The volumetric impinger described has the same deviations from the perfect atmospheric sampler as the human respiratory system. Just how far they deviate from one another is conjectural at this time. Further refinements in our sampling instruments and techniques are needed to answer these questions.

CONCLUSIONS

A simple, uncomplicated volumetric apparatus, which will run unattended for twenty-four hours a day for months at a time and furnish an unbroken series of hourly air samples, has been described in detail. It offers the following advantages over previous methods:

1. Pollen concentrations for the entire day, or for any hour of the day, are easily determined.
2. The apparatus is simple and requires change of slides only once in twenty-four hours.
3. The volume of air sampled is easily controlled.
4. A conversion factor for pollens of varying size is unnecessary.
5. The relationship of pollen concentration to meteorologic phenomena is easily and accurately determined.
6. This apparatus can serve as a useful research tool.

In addition to the numerous advantages cited, the impinger that we have described overcomes the many defects inherent in our present gravimetric method. We feel that the apparatus and procedures herein described meet all the requirements of the Committee on Aeroallergens of the Research Committee of the American Academy of Allergy for a standard volumetric method of pollen sampling, and we herewith submit them for consideration.

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TIETZE'S SYNDROME (PAINFUL NONSUPPURATIVE SWELLING OF COSTOCHONDRAL CARTILAGES)

Report of a Case in a Patient With Bronchial Asthma Treated With Steroids

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THE syndrome of a painful, benign, nonsuppurative swelling of the costochondral or the sternoclavicular junction was first described by Tietze[1] in 1921. This syndrome has been variously called "Tietze's disease," "Tietze's syndrome," "costal chondritis," and "painful nonsuppurative swelling of costochondral cartilages." Up to 1954 only seven cases were recorded in the American literature.[2] With greater recognition since then, however, forty cases of this syndrome have been described from the United States.

Painful, benign, nonsuppurative swelling of the costochondral cartilages is undoubtedly much more common than the 192 cases reported from all parts of the world would indicate. This benign and self-limited syndrome is important to the clinician in that he should be aware of its occurrence in order to be able to differentiate it from the more serious conditions that may produce thoracic pain.

Since many physicians may not be aware of this syndrome, it is very likely that many cases are either unrecognized or unreported. It seems worth while, therefore, to report a typical case of Tietze's syndrome in a patient with asthma and to review the characteristic features of this syndrome.

CASE REPORT

G. B., a 56-year-old white man, was admitted on March 19, 1958, to the medical service of the Maricopa County General Hospital with the chief complaint of severe anterior right-sided chest pain of one day's duration. This pain radiated to the right side of the neck, then to the right shoulder, and later down to the right arm. The pain continued throughout the night, and in the morning the patient noticed a firm, bulbous swelling, the size of a walnut, in the anterior portion of the right second costochondral junction. This swelling was very tender. It was very painful to palpation. There was no local heat or erythema on the overlying skin, which was freely movable. There was no evidence of any systemic or local infection. The patient was afebrile. There was no history of trauma to this area. The pain was aggravated upon movement of the right arm and shoulder and during coughing or deep inspiration. The pain, prior to the administration of steroids, was of such intense severity as to require Demerol or Codeine every three hours for relief.

Past history revealed initial onset of asthma in 1954. The patient had experienced seasonal pollinosis for the past eight years. There was no history of skin allergy, headache,

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migraine, or serum sickness. Ingestion of pork or watermelon caused vomiting. The patient was highly sensitive clinically to house dust, dog dander, and insecticides. No family history of tuberculosis, rheumatic fever, diabetes, or bronchial asthma was elicited.

Laboratory studies revealed a hemoglobin of 12.5 grams; a sedimentation rate of 28 mm. per hour; a white blood count of 7,500; and a normal urinalysis. Roentgenograms of the chest showed a moderate degree of emphysema. No abnormalities were noted in the ribs or at the site of the costochondral junction. Repeat roentgenograms a month later, including special details for ribs, revealed no evidence of a fracture or separation of the cartilages of the costochondral junction.

On the night following admission, the patient developed an acute paroxysm of bronchial asthma. He was treated with intravenous fluids, aminophylline, bronchodilators, and iodides. He remained in status asthmaticus despite adequate hydration and conventional supportive therapy. He was then given hydrocortisone intravenously, with marked symptomatic relief of asthma. He also noted that his painful swelling at the second right costochondral junction was dramatically relieved by administration of hydrocortisone. He was then maintained on oral prednisone, with continued relief of pain. He required no further pain-killing drugs. His chest pain subsided completely in four days.

DISCUSSION

The cause of Tietze's syndrome is unknown. It has occurred in patients of both sexes and of all age groups, from the ages of 11 to 79 years. The mean age is about 30 years. Its most frequent occurrence is in the third decade of life. There is no occupational preference. There is no evidence that it is related to any nutritional deficiency or to pulmonary tuberculosis. The trauma of coughing is a common precipitating factor. Other factors are external trauma, distant focus of infection, acute viral and bacterial infections of the respiratory tract, common colds, bronchitis, and pneumonia. However, whether it is due to the association of infection, toxic factors, or the trauma of coughing is unknown. The most recent etiological theory suggests that "microtrauma" or rheumatoid disease of the interarticular sternocostal ligament may be a causative factor.[3]

The interarticular sternocostal ligament, an inconstant structure, is a strong fibrocartilaginous layer that passes within the joint from the second costal cartilage to the fibrous substance between the manubrium and the body of the sternum. As Motulsky and Rohn[3] point out, the presence of this ligament, most commonly found at the second costal cartilage, rather closely parallels the incidence of involvement of these cartilages by Tietze's syndrome. Another common underlying factor is believed by Raffle[4] to be an unusual distribution of local stresses and strains on the cartilages. Beck and Berkheiser[5] have proposed that the pathologic process consists of a contracture of the ligament lying subjacent to the second costal cartilage, causing the cartilage to buckle forward.

Pathology.—In those cases that were biopsied, histologic examination did not reveal any specific diagnostic features. Normal cartilage is reported. The second costal cartilage is most frequently involved. The lesions may be either single or multiple. When the swellings are multiple, they may be either unilateral, affecting neighboring cartilages on the same side, or bilateral, involving as many as six cartilages. Frey[6] observes that the second rib on the left may be involved somewhat more frequently than that on the right.

Meantime, Wehrmacher[7] and also Bernreiter[8] emphasize that painful, nonsuppurative swelling of the costochondral cartilages must be differentiated from other causes of thoracic pain. When the pain is on the left side, the patient will frequently seek medical attention because of the possibility of heart disease. When the site of involvement is on another part of the thorax or on the right side, the patient may be concerned with the possibility of cancer of the breast, pleurisy, or some other form of pulmonary pathology. Table I shows the conditions to be considered in the differential diagnosis of Tietze's syndrome as listed by Karon and associates.[9]

TABLE I

I. Diseases of costal cartilage
  A. Neoplasm
    1. Benign
    2. Malignant
  B. Infection
    1. Osteochondritis
    2. Specific infectious agent

II. Neoplasms of thoracic wall
  A. Primary
  B. Metastatic

III. Diseases of breast
  A. Infectious
  B. Neoplastic

IV. Diseases of heart, pericardium, and great vessels

V. Posttraumatic conditions
  A. Painful postfracture callus, especially occult tussive fracture
  B. Intercostal neuritis
  C. Costochondral separation

VI. Pulmonary diseases
  A. Pulmonary embolism
  B. Pneumothorax
  C. Mediastinal emphysema
  D. Pleurisy

VII. Miscellaneous diseases
  A. Rheumatoid arthritis of the second to fifth costochondral joints
  B. Presternal edema of mumps
  C. Hodgkin's disease
  D. Multiple myeloma
  E. Painful xiphoid syndrome
  F. Thoracic wall pain
  G. Herpes zoster

SYMPTOMS

The characteristic complaint of Tietze's syndrome is anterior thoracic pain, which is generally localized at the site of the affected cartilage, but most of the time it radiates into the neck, shoulder, and arm. Some patients complain of severe pain in the chest, while others complain of a sensation of heavy weight pushing in on the chest. Many factors, such as sneezing, deep breathing, coughing, and strenuous activity, may contribute to the intensity of the pain. The duration of the pain may be several hours to several months. Sometimes an attack may recur after several years of quiescence. Exacerbations are

not uncommon. The significant finding is that of a bulbous or fusiform swelling of one or more of the costosternal cartilages. This swelling may involve the soft tissue and bone. However, the overlying skin is freely movable and shows no alteration. The mass is generally very tender. Heat, redness, or other signs of suppuration are characteristically absent. The maximum size of the swelling is reached rapidly, and then it either retains this size or regresses in a few weeks. The location of the pain and the swelling is either the costochondral or the sternoclavicular junction. The most frequently involved site is the second costal cartilage. Multiple lesions are not uncommon.

The diagnosis of Tietze's syndrome will be made more commonly if the clinician considers it in determining the causes of chest pain or swelling of the chest wall. Confirmation of the diagnosis of Tietze's syndrome is by exclusion and a positive surgical biopsy specimen showing normal costal cartilage in a patient with a painful nonsuppurative swelling of one or more costochondral cartilages or of the sternoclavicular junction.

TREATMENT

There is no specific treatment for Tietze's syndrome. Symptomatic measures, such as local heat, analgesics, and reassurance, usually suffice for the mild cases. Procaine infiltration affords only temporary relief. Local infiltration with hydrocortisone has given excellent results in the two patients treated by Celio and Nigst.[10] Our patient was given intravenous hydrocortisone followed by oral prednisone for the relief of status asthmaticus. There was a marked relief of swelling and pain, which subsided in four days. X-ray therapy is not effective. Complete surgical excision of the lesion is not warranted if the clinician is satisfied with his diagnosis.

SUMMARY

A case of painful, nonsuppurative swelling of the costochondral cartilage (Tietze's syndrome) in a patient with bronchial asthma is described. The syndrome is a benign self-limiting disease, usually involving one or more of the costal cartilages. The second costal cartilage is most commonly involved. Pain may persist for a few days to several weeks. The swelling may continue for months. The cause is not known. Cough and nontuberculous respiratory disease frequently precede or accompany this syndrome.

Tietze's syndrome is of clinical interest primarily because of its significance in the differential diagnosis of chest pain. It may mimic pleurisy, angina pectoris, pericarditis, mediastinal emphysema, pneumothorax, a painful callus due to occult tussive fracture, or a neoplasm of the thoracic wall or breast. Relief of pain may be afforded by local infiltration with procaine or hydrocortisone. Pain and swelling rapidly subsided in a patient who was given steroids for the relief of status asthmaticus. The syndrome is not uncommon. More cases will be recognized as the clinician considers it in the differential diagnosis of chest pain and of tumors of the chest wall.

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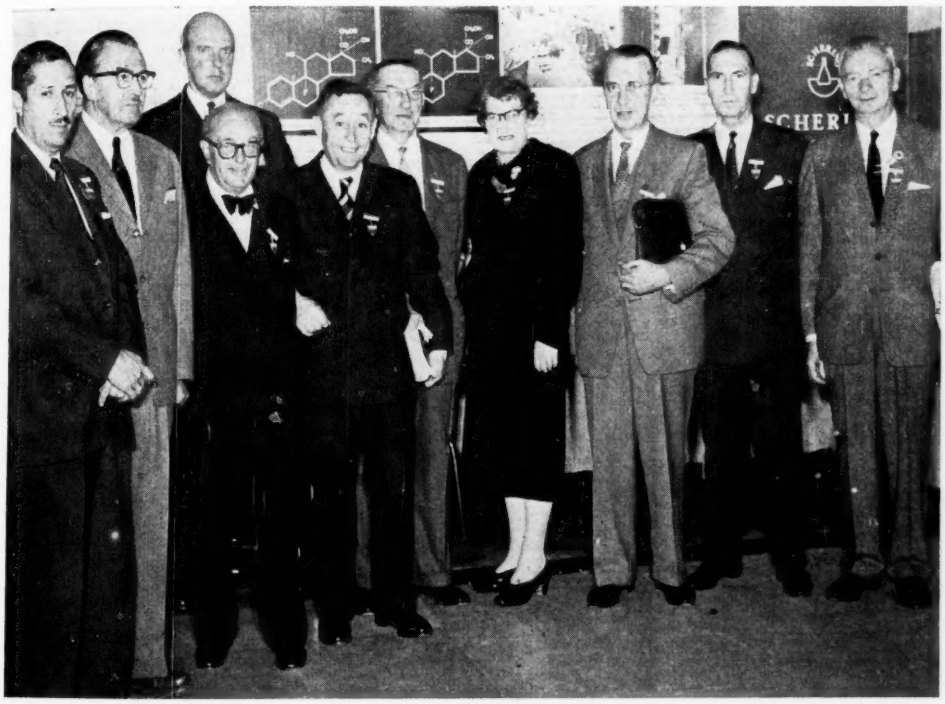
Erratum

The article by Wilhelm, Fisher, and Cooke, which began on page 493 of the November-December, 1958, issue of the JOURNAL, should have borne the title "Experimental Depletion of Mononuclear Cells for the Purpose of Investigating Allergic Contact-Type Reactions." In the second paragraph on page 496, the first sentence should read: "Seven days after the epicutaneous testing, five guinea pigs were injected intracardially with 1 ml. of anti-MC serum, and seven guinea pigs were injected with normal serum." On page 500 the last line of the first paragraph of the discussion should read: ". . . Inderbitzin[7] has demonstrated for the tuberculin reaction." On page 501 the first sentence of the second paragraph should read: "At the time of epicutaneous testing a depletion of eosinophils was observed in all animals injected with either serum."

Announcements

Third International Congress of Allergology

The Third International Congress of Allergology was held recently (Oct. 19 to 26, 1958) in Paris, under the auspices of the International Association of Allergology. The many Americans who attended were enthusiastic over the quality of the extensive scientific program, particularly the symposia. The French hospitality was superb, and the registration exceeded the combined total of the first two Congresses. The symposia, published in a 940-page *Proceedings* (published by Editions Médicales Flammarion, Paris, at the price of $15.00), comprises a résumé of the most important advances in allergy in recent years. Many of the articles are in English, and each has an extensive English summary. The next Congress will be held in New York in 1961.



Executive Committee of International Association of Allergology elected at the Third International Congress of Allergology in Paris. This committee will function until the end of the next Congress, which will he held in New York in 1961.

Left to right: José Cortes (Mexico); E. Letterer (Germany), third vice-president; J. W. Quarles van Ufford (Holland); C. Jimenez-Diaz (Spain), president-elect; B. N. Halpern (France), president; M. J. Gutmann (Israel); Vera Walker (Great Britain); C. H. Walton (Canada), secretary-general; E. Bruun (Denmark), treasurer; S. M. Feinberg (United States), ex-president.

J. M. Quintero Fossas (Cuba), first vice-president, and O. R. Withers (United States), second vice-president, were not present when this photograph was taken.

Obituary

ROBERT CHOBOT

ROBERT CHOBOT died suddenly of a coronary attack in the Claridge Hotel in London, England, on Sept. 26, 1958. Dr. Chobot was born in 1901.

He was graduated from Columbia University in 1920, and four years later he received the Doctor of Medicine degree from the College of Physicians and Surgeons in New York City.

He was among the earliest physicians to become interested in allergy, and in 1926 he was appointed to the staff of the original allergy clinic, then working at the New York Hospital. This connection was continued after the clinic was moved to the Roosevelt Hospital, and for the last six years he was an associate attending on the allergy service of the medical staff at Roosevelt Hospital.

In addition to the valuable service he rendered here, Dr. Chobot was the Director of Pediatric Allergy at University Hospital and Associate Clinical Professor of Pediatrics at the New York University Postgraduate Medical School. He also served as consultant in allergy at the Downtown Beekman Street Hospital. Dr. Chobot was a member of the county and state medical societies, a Fellow of the American Medical Association, a Fellow of the New York Academy of Medicine and the American College of Physicians, and an honorary Fellow of the Allergy Society of the Argentine Republic.

His recognition as a leader in his chosen field is further attested to by the fact that he was selected to be the first president of the American Academy of Allergy at the time of the amalgamation of the Eastern and Western branches of the two allergy societies then existing. He was a diplomate of the Board of Internal Medicine for internal medicine and for allergy.

Dr. Chobot made many contributions to allergy, both in the clinical field and in clinical research. Especially important is his book entitled *Pediatric Allergy,* published by the McGraw-Hill Book Company in 1951. He earned for himself a very high place among his confreres, not only for his medical accomplishments but for himself as a man of attractive personality, for his sound thinking, for his frankness, and for those wonderful qualities that endeared him to his many friends and to his patients as well.

He leaves his widow and his daughter, Barbara (Mrs. Harold K. Deutch). His sudden passing has created a sorrow in the lives of his many admirers that time alone may soften.

R. A. C.